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A STUDY OF ASEPTIC MAINTENANCE BY PRESSURIZATION

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16. Abstract

Studies were performed to determine the pressure differentials required to prevent the passage of airborne microorganisms through holes in membranes acting as barriers to preserve asepsis. Analytical and experimental results showed that the effectiveness of the pressure differential is a function of the particle density, velocity and diameter. At a given particle density, it was observed that a differential pressure of .05 inches of water was sufficient to prevent passage of particles of up to 10 microns in diameter through holes of diameters up to 3000 microns when particle velocities were due only to gravitational forces. At greater particle velocities the dependence on hole diameter became more noticable. A pressure differential .05 inches of water above the stagnation pressure was discovered to be sufficient to maintain asepsis with holes up to 1000 microns in diameter when particle velocities were less than 15 mph. Holes 20, 200, and 1000 microns in diameter were found to be associated with critical velocities of 30, 26 and 15 mph respectively, above which a marked increase in pressure differential was required to prevent penetration. Effects of temporary pressure loss were also evaluated to determine minimum penetration time. A data summary combines the results of this contract with those of the previous studies.

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GLOSSARY OF TERMS

P	=	gas pressure
ρ	=	density
T	=	absolute temperature
R	=	the gas constant
M	=	mass of the gas
V	=	volume of the gas
A_H	=	cross sectional area
P_∞	=	exit pressure
γ	=	ratio of specific heat capacities of gas
d_H	=	orifice diameter
L_H	=	orifice length
μ	=	fluid viscosity
v_H	=	efflux velocity
r_s	=	spore radius
M_s	=	spore mass
v	=	orifice velocity
F_d	=	drag force
x	=	distance along jet axis from orifice exit
t_o	=	time to vent to zero
t_p	=	penetration time

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1.0 SUMMARY

A study of aseptic maintenance by pressurization has been conducted for the Langley Research Center of the National Aeronautics and Space Administration under Contract NAS 1-10061. The study consisted of three phases. The first phase encompassed experimental work which increased the confidence limits of the data previously obtained from the AMP I and AMP II contracts. Results of these studies have demonstrated that a pressure differential as small as .05 inches water pressure above ambient across a membrane separating two quiescent chambers could prevent passage of microorganisms through a single hole against that pressure gradient. Fifty milligrams of lyophilized B. subtilis var. niger spores were aerosolized into a chamber and were presented by gravity to the hole at the bottom of the chamber (quiescent testing). The holes, 1000 to 3000 microns in diameter, were made in .012 inch thick membranes of aluminum. The lyophilized spores which penetrated the hole were captured on an agar medium located at the bottom of the gas chamber, and were allowed to grow into visible colonies.

After establishing the 3000 micron hole as the maximum hole size that could be employed in the test membrane without excessive evaporation from the culture tube media when oxyethylene docosanol was used to retard this evaporation, forty-six tests were performed in the quiescent test apparatus using the test procedures developed during the AMP I and AMP II contracts. These tests were run under the following conditions:

a) Five tests with holes of approximately 1000 microns in diameter, and 0.5 inches differential pressure. No violation of sterility.

b) Three tests with holes of approximately 1667 microns in diameter, and 0.5 inches differential pressure. No violation of sterility.

c) Three tests with holes of approximately 1887 microns in diameter and 0.5 inches differential pressure. No violation of sterility.

d) Ten tests with holes of approximately 1000 microns in diameter and 2.0 inches differential pressure. No violation of sterility.

e) Twenty-five tests as follows:

Seven tests with holes 2000 microns in diameter and 0.5 inches differential pressure. No violation.

Eight tests with holes 2333 microns in diameter and 0.5 inches differential pressure. No violation.

Six tests with holes 3000 microns in diameter and 0.5 inches differential pressure. No violation.

Four tests with holes 3000 microns in diameter and 0.5 inches differential pressure. No violation.

The data obtained under quiescent conditions substantiate the theory that low pressure differentials will effectively prevent passage of microscopic particles through a microscopic hole as long as the atmosphere on either side of the hole is quiescent. The experimental data demonstrated that for all the hole sizes tested, 0.5 inches of water pressure effectively stopped all the particles that challenged the holes. Experimental conditions restricted, somewhat, the particle sizes which challenged the various-sized holes. Theoretical analysis has shown, however, that 0.5 inches of water pressure will effectively stop particles up to 200 microns in diameter, under quiescent conditions.

The second phase of the contract, which ran concurrently with the quiescent tests, dealt with the high velocity particle tunnels, in an effort to more precisely define the relationship between particle velocity and the pressure differential required to maintain asepsis. These experiments were performed in three unique wind tunnels specifically designed and constructed for the AMP II contract.

Theoretical gas flow analysis has clearly demonstrated that the presence of an internal pressure slightly above the flow stagnation pressure will prevent the migration of one to three micron diameter particle. Larger pressure differentials are required to prevent passage through larger holes as the particle size and velocity increases.

A total of 50 tests were run with hole sizes of 20 to 1000 micron in diameter at pressure differentials ranging from .05 to 3.8 inches. These were run at wind velocities ranging from 10 to 30 miles per hour. The test regimes were as follows:

Hole Size In Micron	Air Velocity MPH	Number of Tests
1000	20	3
1000	25	3
1000	30	4
200	10	4
200	15	4
200	20	4
200	25	4
200	30	3
20	10	4
20	15	5
20	20	4
20	25	5
20	30	3

The test procedures used during the course of these experimental studies were those developed during the AMP I and AMP II contracts. Results of the tests run with holes 20, 200 and 1000 microns in diameter indicated that critical velocities associated with these holes were 30, 26, and 15 miles per hour, respectively. As the wind velocities increased beyond these points, a marked increase in pressure differential was required to prevent particle penetration.

The third phase of the experimental studies involved the performance of thirty-five tests in a modified quiescent test apparatus to determine the minimum time required for penetration of a hole by microorganisms on the occasion of loss of pressure differential. The quiescent test chambers were modified to control the pressure differential at two inches of water for a given time

period, then to permit the pressure to vent to zero and hold for a given time period. The two inches of water differential pressure was then reestablished and the cycle repeated every thirty minutes for the duration of the test.

Experimental test studies run with 100, 200 and 1000 micron holes indicate that the minimum time required for penetration of a hole, once the pressure differential has reached zero, is in the order of one second. On occasion, a time span as short as 0.2 seconds after ambient was reached was sufficient to allow penetration. With the smallest hole size (20 μ), on the other hand, 30 to 60 seconds were necessary to achieve penetration probably due to the less frequent challenge of the small orifice by viable particles.

The overall test results indicate clearly a particle size dependence in all the hole sizes, thus necessitating a rigid control on particle sizes and velocities when the protection of holes is to be accomplished with 2.0 inches of water pressure. In general, the use of pressure differentials offers a workable solution to maintenance of sterility after terminal sterilization has been accomplished.

2.0 INTRODUCTION

2.1 BACKGROUND

The program entitled "Aseptic Maintenance by Pressurization" addresses the problem of maintaining the sterility of an extra-terrestrial vehicle by providing a positive pressure differential across the wall of the enclosure used as a barrier against recontamination from viable organisms.

For many years the control of air movement has been employed in hospital, industrial, and pharmaceutical practices to aid in excluding microorganisms from clean rooms or semi-closed systems where it is desirable to maintain a low population density of microorganisms or to prevent the spread of pathogenic organisms from a room or enclosure. The use of a positive pressure differential to achieve an outward gas flow through a leak - microscopic or otherwise - has been adopted by investigators in developing techniques to maintain sterility in closed systems on the premise that such methods will insure the biological integrity of a system. The success of such facilities in maintaining sterility of a system has been well documented. The actual benefit accrued from such a facility, however, has not been established quantitatively, nor has the magnitude of the ΔP required been determined.

Theoretical computations performed for AMP I and AMP II indicated that the establishment of a positive pressure differential can be an effective deterrent to microorganisms tending to pass through small holes. This was largely confirmed in AMP I and AMP II experimentally, but additional data points were required to provide a statistical base to permit calculation of the probability of contamination of a sterile space.

The intent of the present program was to expand the data base provided by the previous contracts, and to obtain data on additional phenomena involving the use of positive pressure to maintain sterility. In so doing, the program evaluated the influence of several parameters, foremost of which were the hole diameter, membrane thickness, pressure differential, wind velocity, and minimum penetration time on the occasion of loss of pressure differential.

2.2 Program Formulation

The AMP III program was formulated to extend the testing of the AMP I and AMP II contracts which investigated the pressure differentials required to prevent the passage of microorganisms under quiescent and non-quiescent conditions, and to determine a minimum penetration time in the event of a pressure loss. The first phase, the quiescent work, was predominantly a continuation of the previous AMP contracts since the design studies and the analytical studies of AMP I and AMP II were applicable. The test apparatus, test protocol and techniques were carried over from the two previous programs with two exceptions: the use of oxyethylene docosanol as an evaporative retardant, and, a spray nozzle for microbial dispersion.

Early in the AMP III program, tests were performed to establish the maximum hole size that could be employed in the test membrane without excessive evaporation from the culture tube media using a dilute emulsion of the wax, oxyethylene docosanol. This non-toxic, stable inert compound, in a 0.2% emulsion greatly extends the useful life of agar in conjunction with apparatus designed for use with continuous air flow. This property of O.E.D. is attributable to its ability to form a monomolecular layer which prevents evaporation from the agar. It has been found that colony growth was not adversely affected due to the addition of this compound. The conclusion of this work was that the use of this compound allows for extended ability to collect and grow microorganisms during the 72 hours of the test run.

In addition, the aerosol dispensers used previously were replaced by an atomizer capable of distributing more uniformly the viable microorganisms within the chambers. The flat fan type spray pattern employed increased the number of particles within the chamber and decreased the size of these particles.

The second phase of the program, the high velocity testing, continued the experimental studies of AMP II. The fifty high velocity tests were performed in the wind tunnels exactly as those of the AMP II contract to more precisely defin e the relationship between particle velocity and pressure differential. To maintain a constant procedure, a detailed checkout form was used on each test run to record the steps as performed. As in the previous program, the methods for dispersion of aerosol, culture tube incubation, air line filters, aerosol head preparation, and pretest checkout, were similar to the quiescent test program. The 50 mg of viable spores aerosoled into the wind tunnel provided a microbial

challenge several orders of magnitude more severe than that found in the quiescent tests.

In addition, the precise geometry of the holes employed in the above tests were determined where possible by the cast technique developed during the previous contracts.

The third phase of the program dealt with microbial penetration under conditions encountered by a loss of pressure differential. This aspect of the work required both analytical and experimental activities in its performance, foremost of which was the modification of the quiescent chambers to allow the pressure to vent to zero, maintain itself there for a precalculated time period, then reestablish itself at two inches Δ P.

Before this was done however, an analytical quantification to predict spore penetration times associated with transient pressure loss was undertaken. In the analyses, it was determined that the minimum spore penetration time was dependent on the following quantities: the volume of air supplying the membrane hole; initial oven pressure across the membrane; external pressure; membrane thickness; hole diameter; acceleration due to gravity; air temperature and viscosity; and spore density and radius. The results of these studies were then used to identify the necessary test parameters and their variations in magnitude.

3.0 ANALYTICAL STUDIES

3.1 SPORE PENETRATION TIMES ASSOCIATED WITH TRANSIENT PRESSURE LOSS

In this analysis, it is determined that the minimum spore penetration time is dependent on the following quantities: the volume of air supplying the membrane hole(s); initial overpressure across the membrane and external pressure; membrane thickness; hole diameter or aggregate area, for more than one hole; acceleration due to gravity; air temperature and viscosity; and spore density and radius. The analysis, although general in nature, is limited to the condition where the initial overpressure is small compared to the external pressure. Depending upon the ratio of membrane thickness to hole diamter, the efflux velocity is predicted by either orifice type flow or Hagen-Poiseuille type flow. Spore penetration times for an initial overpressure of 2" H_2O and a membrane thickness of 305 microns are provided for hole diameters of 20, 200 and 1000 microns, spore radii of 1 and 15 microns and volumes between 1 and 10^5 cc. Examination of these results shows that, for orifice type flow (membrane thickness to hole diameter ratio less than 5), the spore penetration time is most sensitive to the hole diameter and spore radius (squared dependence), and relatively insensitive to the initial overpressure (square root dependence). The same dependences apply also for the Hagen-Poiseuille type flow except for the hole diameter (quartic dependence) and initial overpressure (linear dependence).

By combining the equation of state for an ideal gas,

$$P = \rho RT \tag{1}$$

in which P denotes the gas pressure, ρ the density, T the absolute temperature, and R the gas constant, with the density relation for a homogeneous mixture,

$$\rho = M/V \tag{2}$$

in which M and V denote the mass and volume of the gas, the pressure can be expressed as:

$$P = RTM/V \tag{3}$$

If the gas is allowed to exhaust through an orifice while the gas temperature and volume are kept constant, the time rate of change of the pressure is obtained by differentiating Equation 3,

$$\frac{dP}{dt} = \frac{RT}{V}\frac{dM}{dt} \tag{4}$$

For the steady flow of an ideal gas through an orifice of cross-sectional area A_H against an exit pressure P_∞ , the mass flow is given by

$$\frac{dM}{dt} = - \frac{P_\infty A_H}{\sqrt{RT}} \left(\frac{2\gamma}{\gamma-1} \left(\frac{P}{P_\infty}\right)^{\frac{\gamma-1}{\gamma}} \left(\left(\frac{P}{P_\infty}\right)^{\frac{\gamma-1}{\gamma}} - 1 \right) \right)^{1/2} \tag{5}$$

so that

$$\frac{dP}{dt} = - \frac{P_\infty \sqrt{RT}\, A_H}{V} \left(\frac{2\gamma}{\gamma-1} \left(\frac{P}{P_\infty}\right)^{\frac{\gamma-1}{\gamma}} \left(\left(\frac{P}{P_\infty}\right)^{\frac{\gamma-1}{\gamma}} - 1 \right) \right)^{1/2} \tag{6}$$

in which δ denotes the ratio of the specific heat capacities of gas which is 7/5 for room temperature air. For convenience define:

$$\epsilon \equiv (\gamma-1)/\gamma \tag{7}$$

$$P \equiv P/P_\infty \tag{8}$$

$$k \equiv \sqrt{RT}\, A_H/V \tag{9}$$

$$W \equiv P^\epsilon \tag{10}$$

This produces

$$\frac{W^2}{(W-1)^{1/2}}\, dW = - \sqrt{4/7}\, k\, dt \tag{11}$$

whereupon integration yields

$$2\,(8 + 4W + 3W^2)\,(W-1)^{1/2}/15 = \sqrt{4/7}\, kt + \text{Const.} \tag{12}$$

Letting

$$P = 1 + \Delta P \tag{13}$$

then, approximately, on condition that ΔP is small compared to unity,

$$W = P^{2/7} \cong 1 + 2\Delta P/7 \tag{14}$$

which when substituted into Equation 12 gives after simplifying

$$\Delta P = 7\,(\text{Const.} - kt/\sqrt{7})^2/2 \tag{15}$$

With the initial condition at

$$t = 0 \tag{16}$$

$$\Delta P = \Delta P_o \tag{17}$$

Equation 15 reduces to

$$\Delta P = \Delta P_o \left(1 - kt/\sqrt{2\Delta P_o}\right)^2 \tag{18}$$

Thus, the pressure drop across the orifice reaches zero when

$$t = t_e = \sqrt{2\Delta P_o}/k \tag{19}$$

By combining the alternate form of the mass continuity equation

$$\frac{dM}{dt} = - \rho v_H A_H \tag{20}$$

with Equation 5 under the condition expressed by Equation 14, the efflux velocity of the orifice may be obtained in the form

$$v_H = \sqrt{2RT\Delta P_o}\ (1 - kt\sqrt{2\Delta P_o}) \tag{21}$$

Equations 18, 19, and 21 are applicable only if the orifice diameter d_H is comparable to its length L_H. If the orifice diameter is considerably less than its length the flow will be similar to Hagen-Poiseuille pipe flow for which

$$v_H = \frac{P_\infty d_H^2}{32 \mu L_H} \Delta P \tag{22}$$

with μ denoting the fluid viscosity. For this case, one obtains the differential equation

$$\frac{d(\Delta P)}{dt} = - \frac{\pi d_H^4 P_\infty}{128 \mu L_H V} \Delta P \tag{23}$$

With the initial conditions the solutions to the above differential equation is

$$\Delta P = \Delta P_o e^{- \frac{\pi d_H^4 P_\infty}{128 \mu L_H V} t} \tag{24}$$

from which the time dependence of the efflux velocity is obtained

$$v_H = \frac{P_\infty d_H^2}{32\mu L_H} \Delta P_o e^{- \frac{\pi d_H^4 P_\infty}{128\mu L_H V} t} \tag{25}$$

In the following derivation, for the case of orifice type flow, Equation 21 is rewritten

$$v_H = v_{Ho} (1 - t / t_e) \tag{26}$$

where

$$v_{Ho} = \sqrt{2 R T \Delta P_o} \tag{27}$$

and, for the case when the hole diameter is considerably less than its length, Equation 25 is rewritten

$$v_H = v_{Hi} \, e^{-t/t_i} \tag{28}$$

where

$$v_{Hi} = \frac{P_\infty \, d_H^{\,2} \, \Delta P_o}{32 \mu \, L_H} \tag{29}$$

and

$$t_i = \frac{128 \mu \, L_H \, V}{\pi d_H^{\,4} \, P_\infty} \tag{30}$$

Consider the problem of a spherical spore of radius r_s and M_s which is approaching an orifice with velocity v and which is acted upon by a gravitational field aligned with the orifice axis and directed inward. Newton's Second Law provides the equation of motion of the spore:

$$M_s \frac{dv}{dt} = M_s \, g - F_d \tag{31}$$

where F_d represents the drag force which for the anticipated conditions will be given by Stokes' Drag Law

$$F_d = 6 \pi \mu r_s \, (v + v_H) \tag{32}$$

Defining

$$\alpha \equiv 6 \pi \mu r_s / M_s \tag{33}$$

and substituting the drag force into the equation governing the spore motion yields

$$\frac{dv}{dt} + \alpha v = g - \alpha v_H \tag{34}$$

Before proceeding to the solution of the above equation, in order to establish the proper initial conditions, a more precise definition of the problem is required. The volume behind the orifice is initially pressurized high enough that penetration of the spores through the orifice cannot occur, that is to say, initially, $\alpha v_H > g$. At time zero the supply pressure is cut off and, as the overpressure across the orifice drops to zero, at some later time t_o the drag and gravity forces acting on a spore within the jet issuing from the orifice will be equal, i.e., $\alpha v_H = g$. At time t_o the spore velocity is zero and its position within the jet will depend on how rapidly the pressure along the jet decays with distance away from the orifice exit. With x denoting the distance along jet axis from the orifice exit, the initial conditions for the problem to be solved are:

At $t = t_o$ (35)

$v = 0$ (36)

$x = x_o$ (37)

where, from Equations 26 and 28 for the two cases of interest, the time is given by

$$t_o = t_e \left(1 - g/\alpha v_{H_o}\right) \tag{38}$$

for the orifice type flow and

$$t_o = t_i \ln \left(\alpha v_{H_i}/g\right) \tag{39}$$

for the Hagen-Poiseuille type flow.

Treating the latter case first, solution for v gives

$$v = e^{-\alpha(t-t_o)} \left(\alpha t_i v_{H_i} e^{-t_o/t_i} \Big/ (\alpha t_i - 1) - g/\alpha\right) + g/\alpha - \tag{40}$$

$$- \alpha t_i v_{Hi} e^{-t/t_i} \Big/ (\alpha t_i - 1)$$

and for x these results

$$x = x_o + g(t-t_o)/\alpha - \left(1 - e^{-\alpha(t-t_o)}\right) g/\alpha^2 \tag{41}$$

$$+ (t_i g/\alpha) \left((\alpha t_i e^{-(t-t_o)/t_i} - e^{-\alpha(t-t_o)}) \Big/ (\alpha t_i - 1) - 1\right)$$

Consequently, the time t_p required for a spore to penetrate the membrane is the value which satisfies

$$L_H - x_o = g(t_p - t_o)/\alpha - \left(1 - e^{-\alpha(t_p - t_o)}\right) g/\alpha^2 \tag{42}$$

$$+ (t_i g/\alpha)\left(\left(\alpha t_i e^{-(t_p - t_o)/t_i} - e^{-\alpha(t_p - t_o)}\right)/(\alpha t_i - 1) - 1\right)$$

Under the conditions

$$\left(2 t_i \left(\alpha + \alpha^3 (L_H - x_o)/g\right)\right)^{1/2} >> 1 \tag{43}$$

$$\alpha t_i >> 1 \tag{44}$$

$$\left(2\left(1/\alpha + \alpha (L_H - x_o)/g\right)/t_i\right)^{1/2} < 1 \tag{45}$$

Equation 42 may be reduced to a form which can be inverted to get an approximate relation for the spore penetration time:

$$t_p \cong t_o + \left(2 t_i (1) \alpha + \alpha (L_H - x_o)/g\right)^{1/2} \tag{46}$$

To determine the penetration time in the case of orifice type flow, the resulting solution for v is

$$v = e^{-\alpha(t - t_o)}\left(v_{Ho}(1 + 1/\alpha t_e) - v_{Ho} t_o/t_e - g/\alpha\right) \tag{47}$$

$$+ g/\alpha - v_{Ho}(1 + 1/\alpha t_e) + v_{Ho} t/t_e$$

and for x one obtains

$$x = x_o + g(t - t_o)/\alpha + v_{Ho}(t^2 - t_o^2)/2t_e \tag{48}$$

$$- v_{Ho}(t - t_o)(1 + 1/\alpha t_e) + v_{Ho}\left(1 - e^{-\alpha(t - t_o)}\right)/\alpha^2 t_e$$

It must be noted that Equations 47 and 48 are valid only if $t \leq t_e$ and, for this condition, the time required for a spore to penetrate the membrane is the value which satisfies

$$L_H - x_o = g(t_p - t_o)/\alpha + v_{Ho}(t_p^2 - t_o^2)/2t_e \tag{49}$$

$$- v_{Ho}(t_p - t_o)(1 + 1/\alpha t_e) + v_{Ho}\left(1 - e^{-\alpha(t_p - t_o)}\right)/\alpha^2 t_e$$

Under the condition

$$t_e \, g / \alpha \, v_{Ho} \qquad < 1 \tag{50}$$

Equation 49 may be reduced and inverted to get an approximate expression for the spore penetration time:

$$t_p \cong t_o + \left(2 t_e \, (L_H - x_o) / v_{Ho} \right)^{1/2} \tag{51}$$

If the value of t_p obtained from Equation 51 exceeds t_e then the value of which satisfies Equation 49 will also exceed t_e and the spore penetration time must be obtained as follows. For $t > t_e$ the governing Equation (34) becomes

$$\frac{dv}{dt} + \alpha v = g \tag{52}$$

and the initial conditions are provided from Equations 47 and 48 at $t = t_e$. With these initial conditions, the solutions of Equation 52 for the spore velocity and coordinate are

$$v = v_{Ho} \left(e^{-\alpha (t - t_o)} - e^{-\alpha (t - t_e)} \right) / \alpha t_e + g / \alpha \tag{53}$$

$$\begin{aligned} x = {} & x_o + g \, (t - t_o) / \alpha + v_{Ho} \, (t_e^2 - t_o^2) / 2 t_e \\ & + v_{Ho} \left(e^{-\alpha (t - t_e)} - e^{-\alpha (t - t_o)} \right) / \alpha^2 t_e \\ & - v_{Ho} \, (t_e - t_o) \, (1 + 1 / \alpha t_e) \end{aligned} \tag{54}$$

The time required for a spore to penetrate the membrane is the value of t_p which satisfies

$$\begin{aligned} L_H - x_o = {} & g \, (t_p - t_o) / \alpha - g \, (1 + t_e g / 2 \, v_{Ho}) / \alpha^2 \\ & + v_{Ho} \left(e^{-\alpha (t_p - t_e)} - e^{-\alpha (t_p - t_o)} \right) / \alpha^2 t_e \end{aligned} \tag{55}$$

With the condition

$$g / \alpha \, v_{Ho} \qquad << 1 \tag{56}$$

Equation 55 may be reduced and inverted to obtain an approximate relation for the spore penetration time:

$$t_p = t_o + \alpha (L_H - x_o) / g + (1 + t_e g/2 v_{Ho}) / \alpha \quad (57)$$

Spore penetration times obtained by numerical solution of Equations 42 and 49 or 55, as the case may be, are provided in Figures 3.1, 3.2 and 3.3 for hole diameters of 20, 200, and 980 microns, respectively. All results were generated for an initial overpressure of 2 inches of water against an external pressure of one atmosphere. In each figure, solutions are shown for spore diameters of 1 and 15 microns with the spore mass density taken equal to that of water (1 gm/cc). In all cases, the spore was assumed located at the external face of the membrane at the instant when the gravitational force and the drag force acting on the spore were equal. Consequently, these computations represent the minimum spore penetration times; however, a more accurate determination of the actual spore penetration times cannot be made without knowledge of the pressure decay along the jet issuing from the hole.

In Figure 3.1 the ratio of hole length to diameter definitely indicates the flow is of the Hagen-Poiseuille type and the solutions are provided from Equation 42. Although not shown, the spore penetration times predicted by Equation 46 are in good agreement with the exact values for the small spore, being 4% low at a volume of 1 cc to within the convergence tolerance of the exact solution at the higher volumes, and, for the larger spore, to within the convergence tolerance over the whole volume range.

In Figures 3.2 and 3.3 for the two larger holes, orifice type flow prevails and solutions are provided from Equations 49 or 55, depending upon which is applicable. For these calculations an orifice discharge coefficient (ratio of actual flow area to orifice area) was employed with the value being 0.6 as ascertained from the experimental pressure-flow results. In all four cases Equation 57 predicts the spore penetration times to within the convergence tolerance (0.01%) of the exact solution over the entire volume range.

The magnitude of the effect of spore location at the instant of force balance on the penetration time is shown in Figure 3.4 for the 980 micron hole - 1 micron spore combination. In this comparison three cases are considered. At the instant of force balance the spore is assumed to be located at the orifice exit, one membrane thickness (0.03048 cm) away from the exit, and five membrane thicknesses from the exit. In all three cases Equation 57 predicts the penetration time to within the accepted accuracy for the exact solutions.

Figure 3.5 is a composite of the three-hole diameters vs. time relationships. The theoretical analysis was initially based on an ideal 20, 200 and 1000μ hole diameter. In actuality, however, hole sizes of 33, 190 and 980μ were employed. As can be seen from the graph, the penetration time for a 1μ particle through a 20μ opening is in the order of 10^3 seconds. The same 1μ particle on the other hand, requires only 5×10^2 seconds to penetrate a 33μ hole on the occasion of pressure decay - an order of magnitude less.

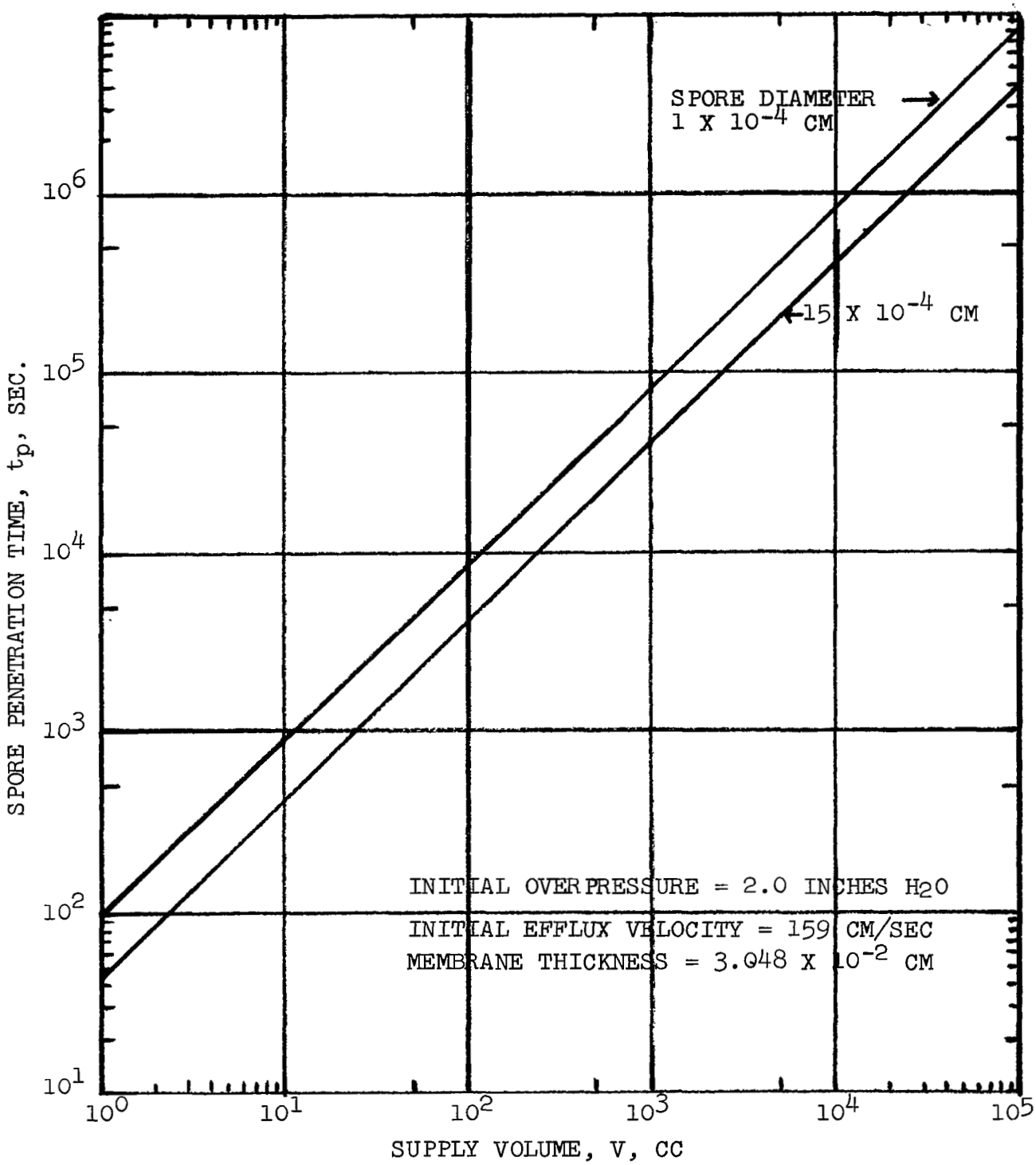


FIGURE 3.1 SPORE PENETRATION TIME VERSUS SUPPLY VOLUME FOR A 20 MICRON DIAMETER HOLE

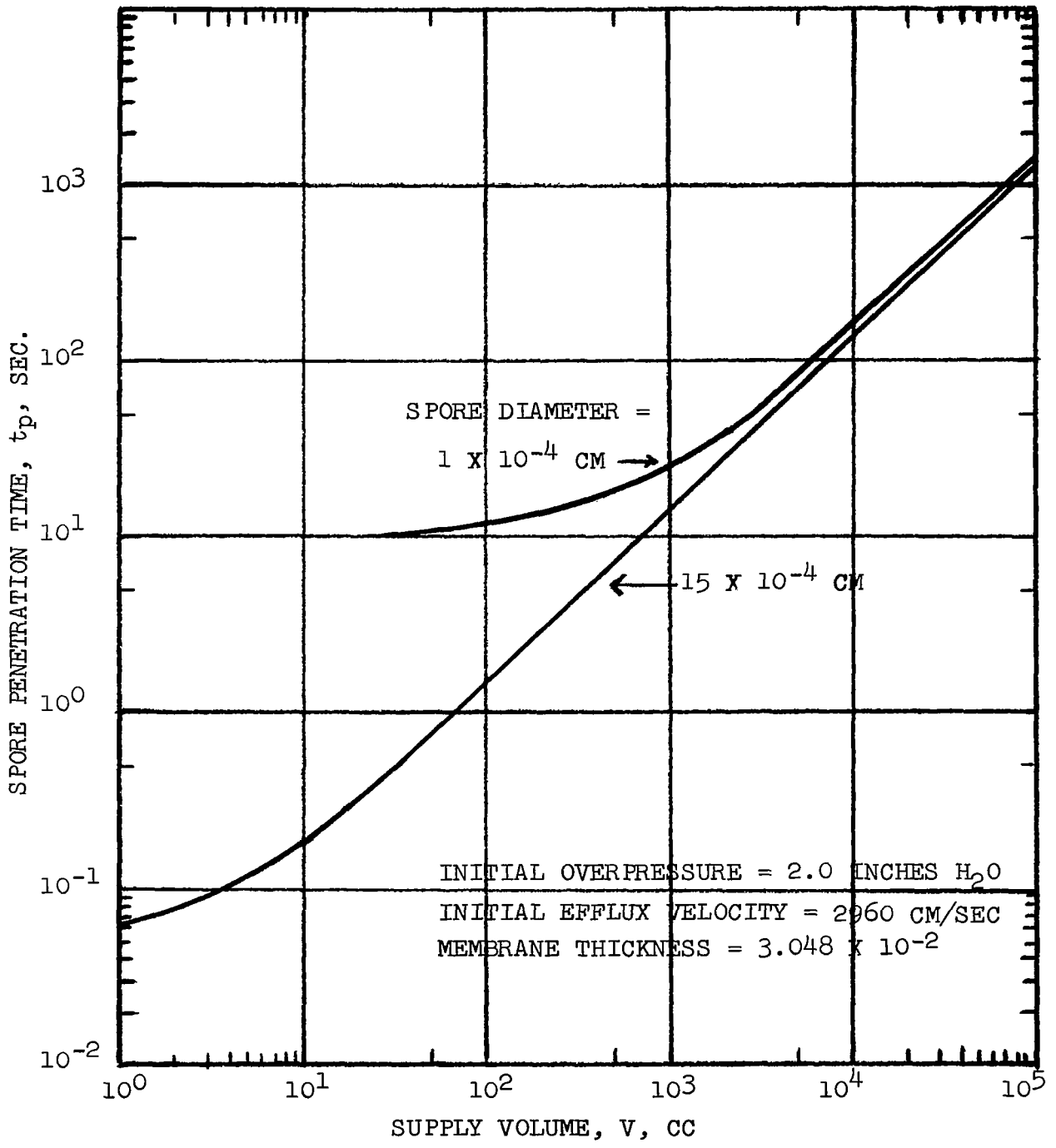


FIGURE 3.2 SPORE PENETRATION TIME VERSUS SUPPLY VOLUME FOR A 200 MICRON DIAMETER HOLE

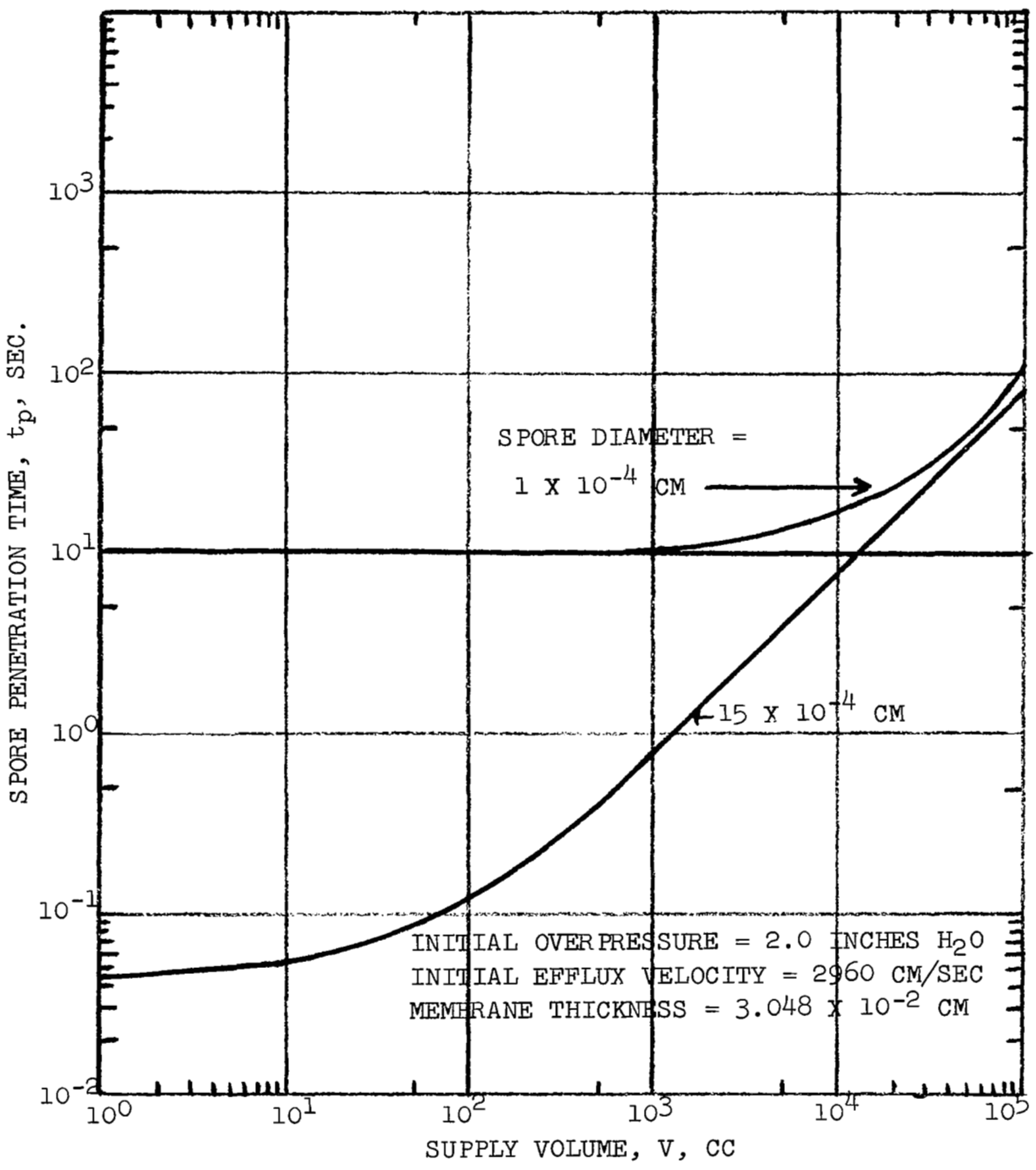


FIGURE 3.3 SPORE PENETRATION TIME VERSUS SUPPLY VOLUME FOR A 980 MICRON DIAMETER HOLE

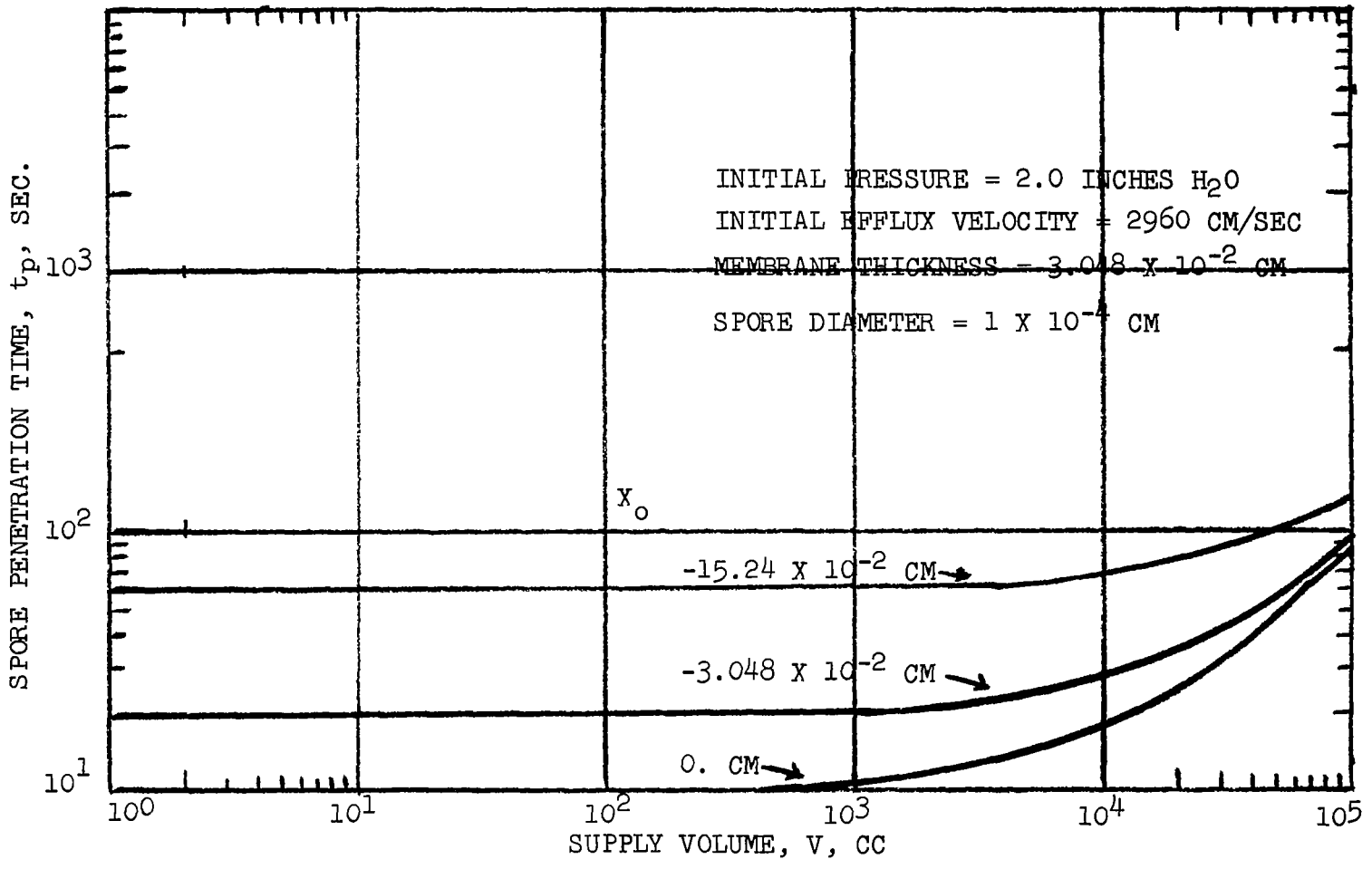


FIGURE 3.4 EFFECT OF INITIAL SPORE LOCATION ON PENETRATION TIME FOR THE 980 MICRON HOLE - 1 MICRON SPORE COMBINATION

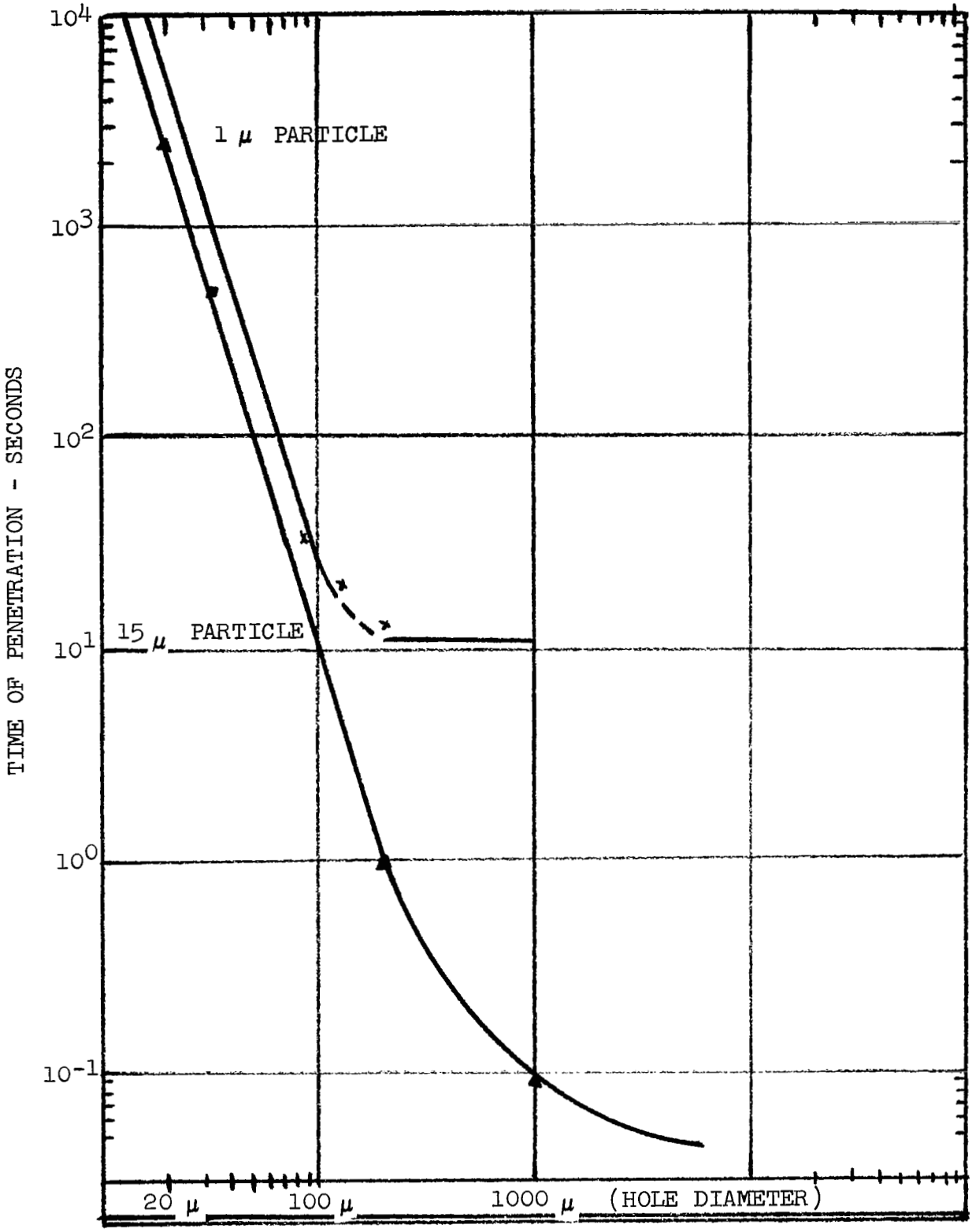


FIGURE 3.5 PENETRATION TIME VS. HOLE DIAMETER - 60 CC SUPPLY VOLUME

4.0 EXPERIMENTAL PROGRAM

4.1 APPARATUS DESCRIPTION

4.1.1 Quiescent Apparatus

The laboratory apparatus for the determination of the flow of microorganisms through various microscopic holes in membranes, and for controlling a range of differential pressures, was designed and fabricated during the course of the AMP II contract. In brief, the apparatus, (shown in Figure 4.1) consisted of a twenty-two liter spherical glass flask into which was aerosoled, through a dispenser head, the lyophilized microorganisms. An agitating fan momentarily circulated the dispersion and was then shut down, allowing the organisms to challenge the membrane hole situated within the membrane holder at the base of the flask. Attached to the holder are air-line filters which maintain the sterility of the air passing across the membrane to a manometer indicating the Δ P across the membrane.

4.1.2 High Velocity Apparatus

Three high velocity wind tunnels designed and fabricated for the AMP II contract, were used to determine the differential pressures required at various wind velocities to prevent penetration through microscopic holes in membranes. Each 6.6 square foot tunnel was adapted with a reversible motor fan which propelled the aerosolized microorganisms past the viable culture chamber located in the restricted leg of the tunnel. The air velocity was monitored by means of a pitot tube connected to a differential pressure gage while the tubing controlling the air flow across the membrane was connected to a pressure transducer which indicated the Δ P in excess of the stagnation pressure. The viable culture tube was located centrally within the restricted leg of the tunnel where the greatest microbial challenge would be encountered. Turning vanes within the tunnels kept the air, flowing past the membrane assembly, laminar. Microorganisms, upon the event of penetration of the membrane hole, grew upon the agar located in a culture tube beneath the membrane hole. These colonies were then detected visibly at the termination of the 72 hour test.

EXPERIMENTAL APPARATUS

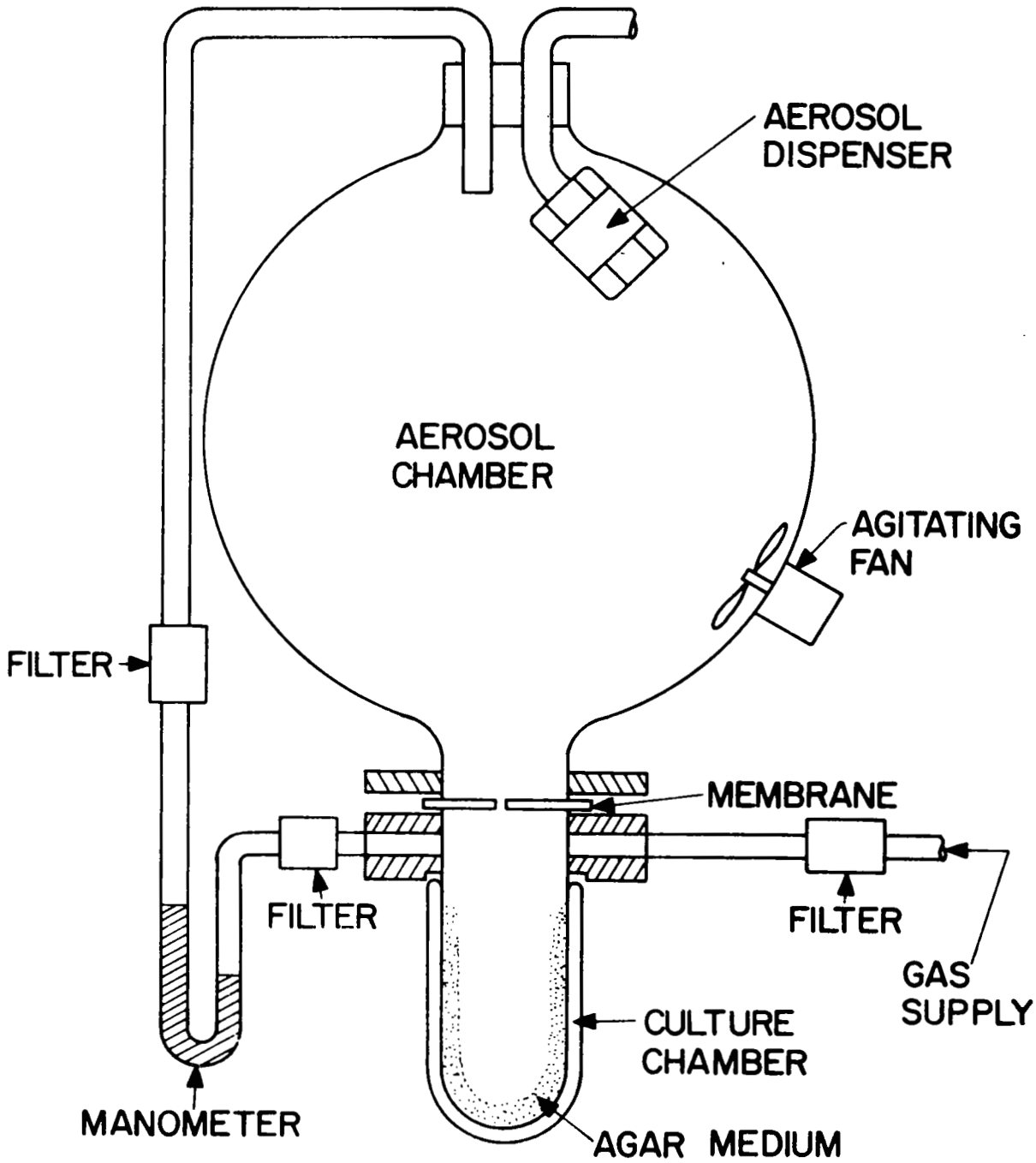
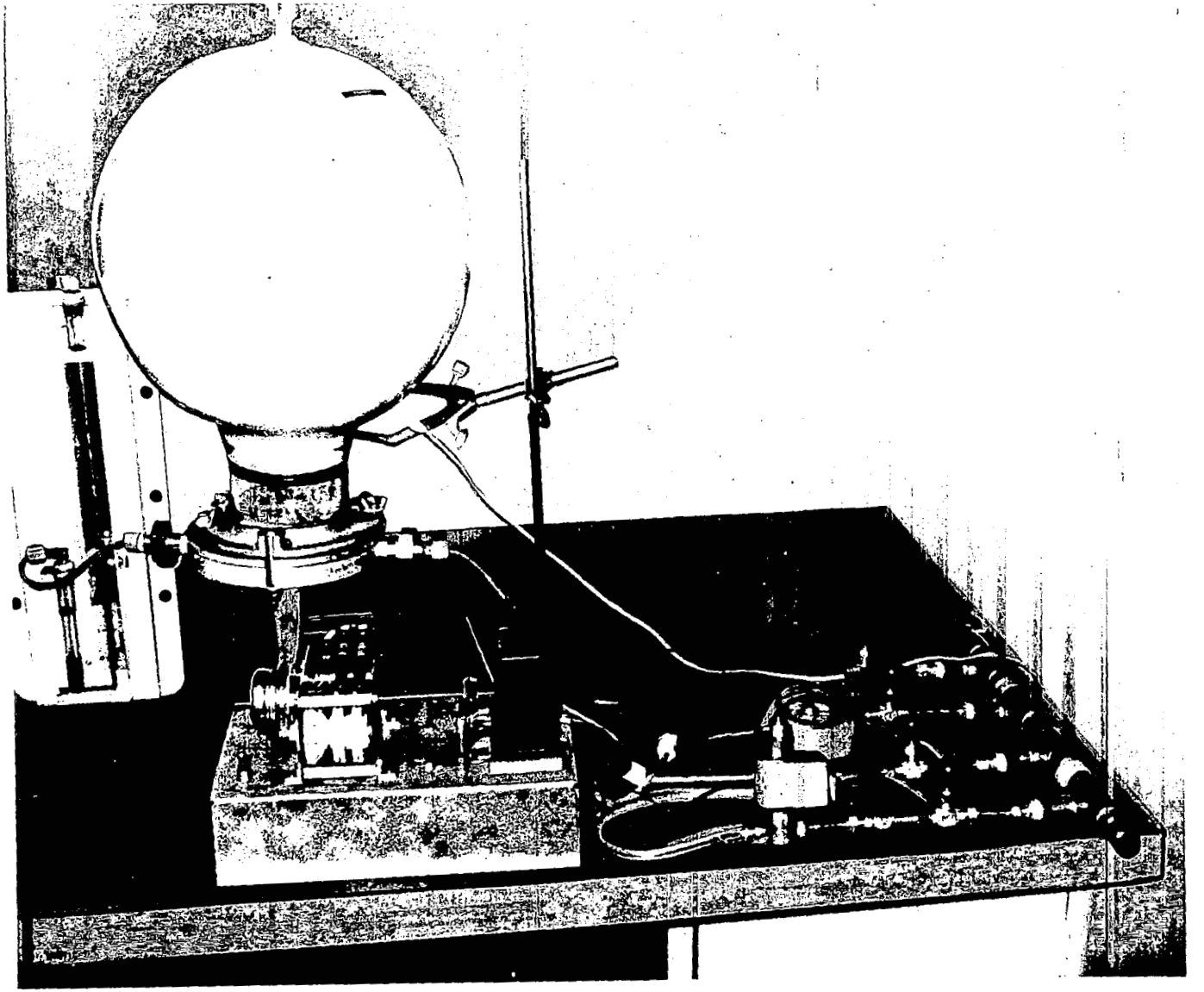


FIGURE 4.1



PHOTOGRAPH P-0 PRESSURE LOSS TEST EQUIPMENT

TEST PROCEDURE - QUIESCENT TESTS

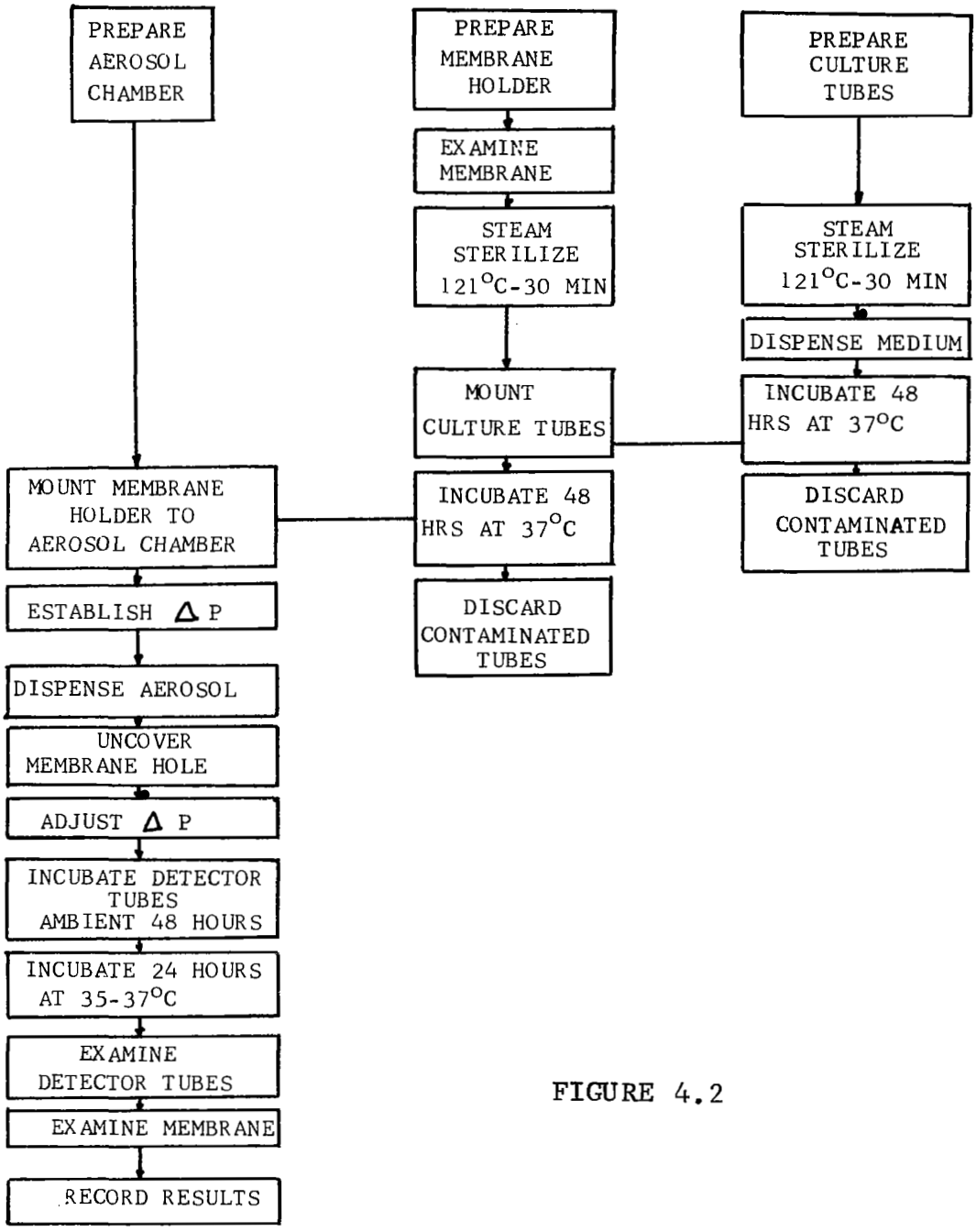


FIGURE 4.2

4.1.3 Quiescent Pressure Loss Test Equipment

An apparatus for determining the minimum time required for penetration on the occasion of loss of pressure differential was designed using the quiescent test equipment as a basic set up. Two units were fabricated and consist of the materials used for quiescent testing, plus the following equipment assembled as shown in Photograph P-0.

Time Interval Meter - The timer used to measure the time interval from the moment the Δ P was shut off until its subsequent reinstatement was a Model 2021A Dual Time Interval Meter, Computer Measurements Company, San Fernando, California.

Time Delay Relay - The timer controls used were Series 319 Adjustable Plug-In Time Delay Relays of the following types: 3 second type 319A34901X; 30 second type 319A006Q1X and 100 second type 319A13401X. Automatic Timing and Controls, Inc., King of Prussia, Pennsylvania.

Program Controller - Adjustable Cam Program Controllers, series 2300B, used to time the 30 minute invervals during the 72 hour test runs, were purchased from Automatic Timing and Controls, Inc., King of Prussia, Pennsylvania.

4.2 TEST PROTOCOL

4.2.1 Quiescent Tests

The test procedures used during the quiescent testing program followed closely the procedures used during the AMP II program, and are shown in the flow diagram in Figure 4.2. Fifty mg of lyophilized B. subtilis var. niger spores were aerosoled into the chamber by means of an air rupture of a seal within the spray nozzle. After further dispersion by the fan, the microbial particles were allowed to settle within the chamber and thus challenge the hole in the membrane protecting the viable culture chamber beneath. A positive air pressure of approximately 0.5 inches water pressure above ambient maintained a steady air flow through the membrane hole, preserving the integrity of the sterile environment within the viable culture tube. Any violation of the system would be noticed during the course of a 72 hour test by the appearance of a microbial colony upon the surface of the agar within the tube. To facilitate growth, the culture tube was incubated at 37°C in-situ for the last 24 hours of each test.

4.2.2 High Velocity Testing

Three tests were run simultaneously in three wind tunnels in a manner similar to that of the quiescent tests. Instead of quiescent chamber conditions, however, a predetermined wind velocity and pressure differential was incorporated into the experiment. The flow sheet shown in Figure 4.3 indicates only slight variations from that of Figure 4.2. One noticeable exception is the absence of a 24 hour incubation cycle at 35-37°C. This was excluded because of the difficulty that would be encountered in incorporating a heating mantle into the wind tunnel. It was felt that the mantle, although a useful tool in growing visible colonies, was not a complete necessity and that equally reliable results could be obtained without it. Photograph P-1 shows the High Velocity Test Apparatus.

4.2.3 Quiescent-Transient Pressure Loss-Tests

The quiescent testing procedure was adapted to the quiescent-transient pressure loss tests by incorporating into the system a series of timers and relays which were capable of automatically controlling the time/pressure variables. The viable culture membrane was put into place by attachment to the chamber. The air pressure line and manometer line were attached and the pressure across the membrane adjusted to 2.0 inches Δ P. After the aerosol was dispersed into the chamber and allowed to settle for 5 minutes, the tape covering the membrane hole was removed and the pressure readjusted to 2.0 inches. The timing sequence was then activated. At 30 minute intervals the Program Controller activated the time delay relay and at the same time closed the solenoid valve supplying air to the system. After the appropriate time interval, the timer opened the solenoid valve and the Δ P was immediately reestablished at 2.0 inches. A time interval meter was connected in parallel with the programmer so that the time lag could be accurately recorded. During the course of the 72 hour test, the membrane hole was challenged approximately 144 times for a predetermined time interval. Any violation in sterility was noticed by microbial colony growth on the agar within the viable culture tube.

TEST PROCEDURE - HIGH VELOCITY

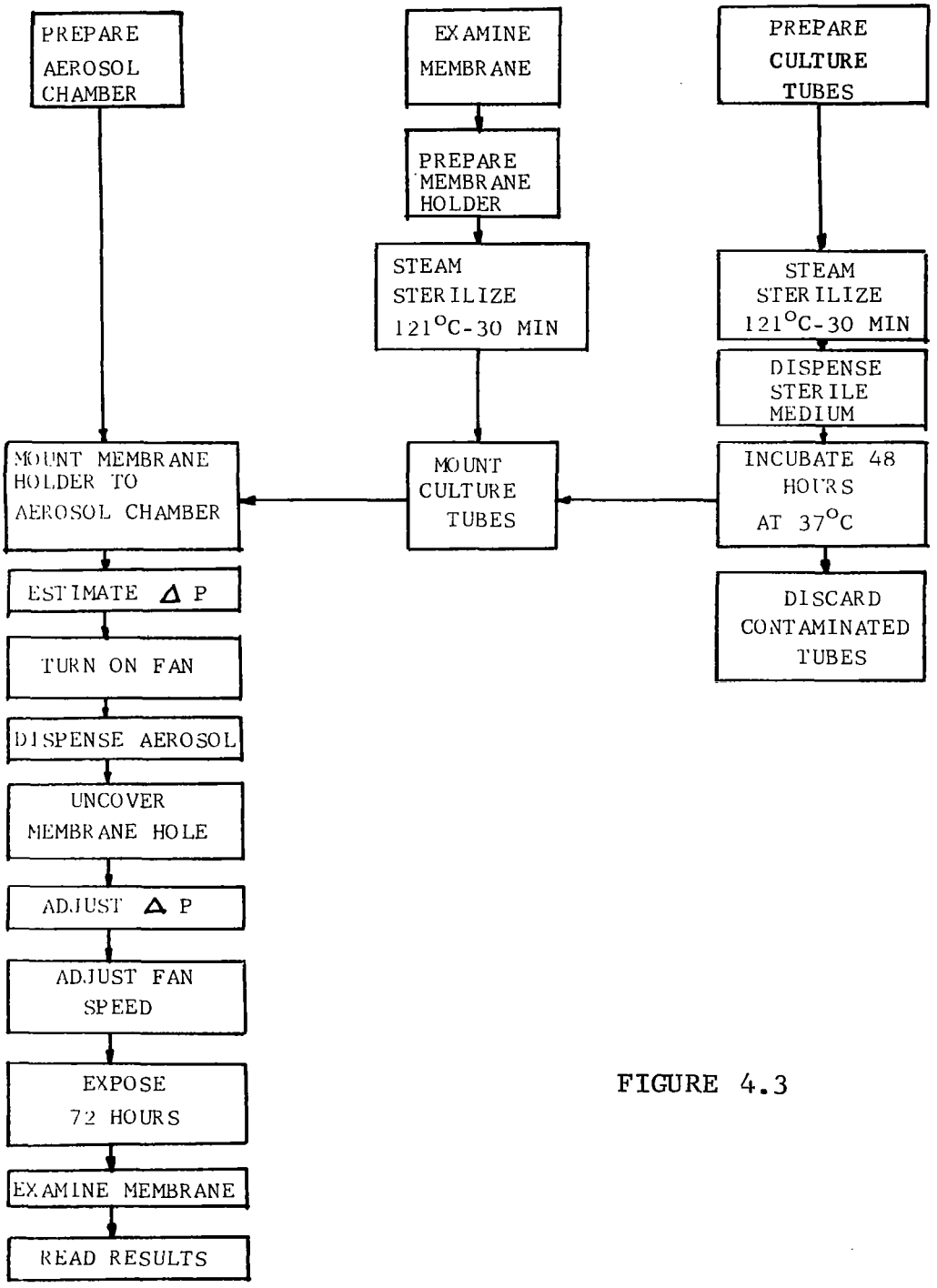


FIGURE 4.3

TEST PROCEDURE - TRANSIENT PRESSURE LOSS

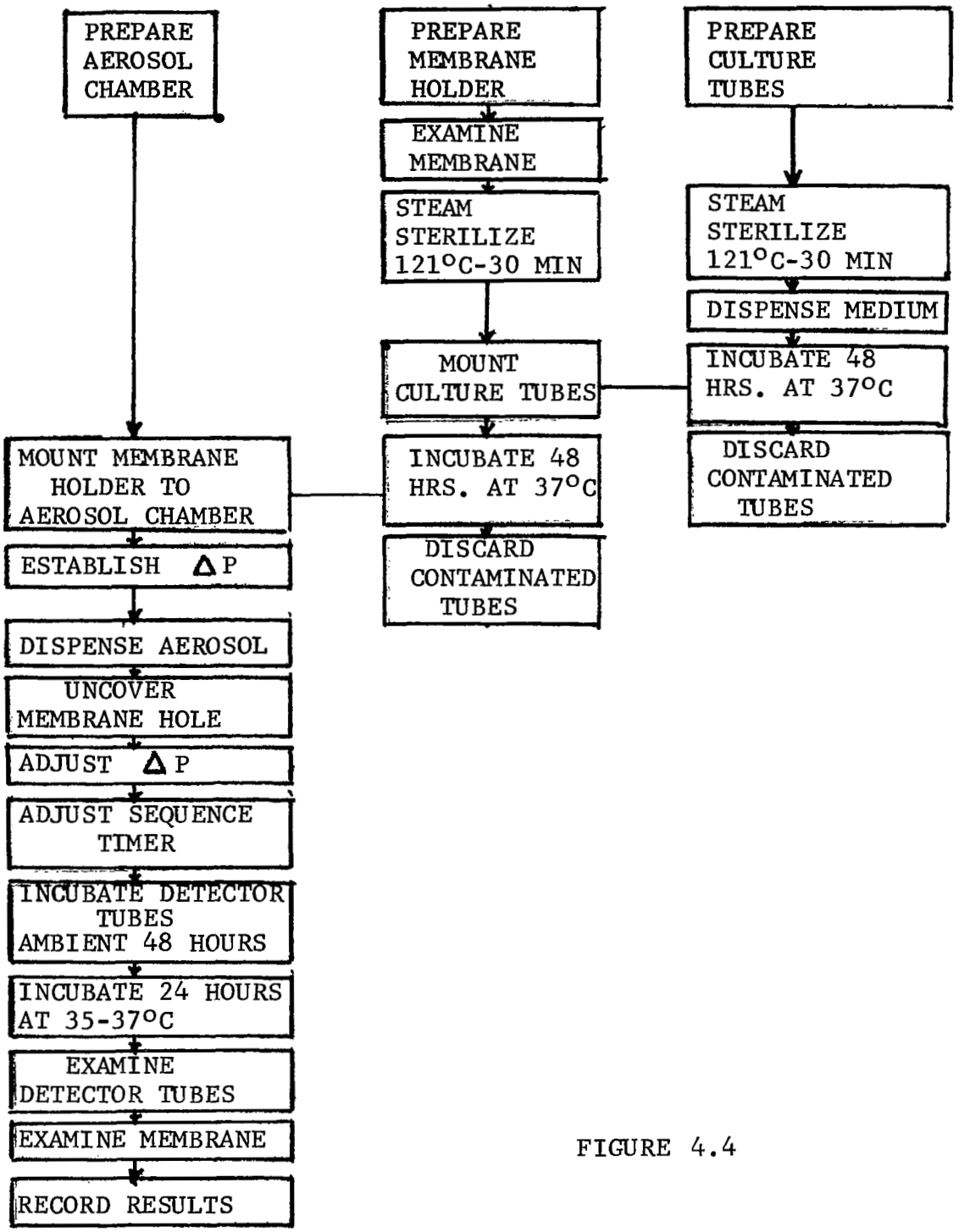
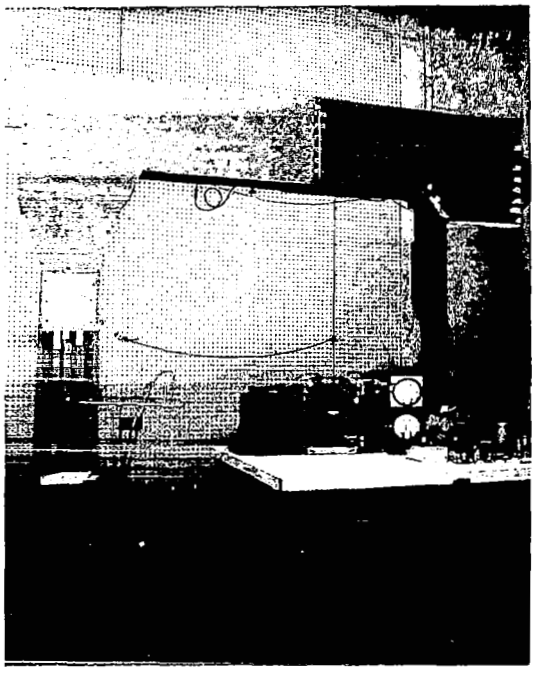


FIGURE 4.4

HIGH VELOCITY TEST EQUIPMENT



PHOTOGRAPH P-1

4.3 HOLE CHARACTERIZATION

Before its incorporation into a particular test run, each membrane hole was examined microscopically and its dimensions observed and recorded. Those membranes considered too large or too small were discarded. In general the hole sizes were no more than ±10% of the size recorded. The one exception was the 20 μ recorded size. Nearly all of these laser drilled holes were 33 μ in diameter at the end facing up into the chamber, and larger at the bottom end. The 20 μ , 100 μ and 200 micron holes were drilled with a laser, while the larger sizes were machine drilled and were more nearly symmetrical.

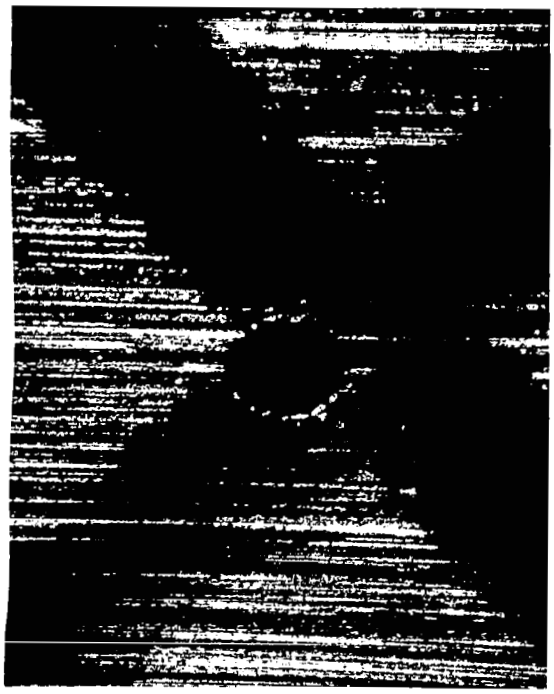
To better characterize the microscopic holes in the aluminum membrane, a destructive technique developed and employed during the AMP II contract was employed. Briefly, an epoxy resin was forced into the hole and allowed to cure. The aluminum was then etched away with a 5% solution of hydrofluoric acid and the remaining resin cast photomicrographed and measured by means of an ocular micrometer. A representative geometry of one of the 200 μ diameter holes was shown to have a top dimension of 221 μ and a bottom dimension of 332 μ . The geometry was that of a truncated cone approximately 304 microns in length, inclined approximately 22° from the verticle. Photomicrograph P-2 is a representative sample of a laser-drilled hole, showing the top and bottom openings made by the laser.

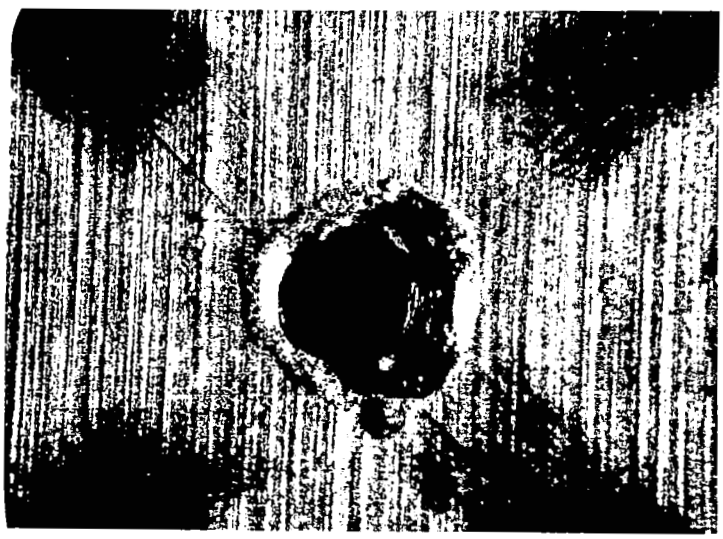
4.4 PARTICLE DISTRIBUTION - HIGH VELOCITY

Several experiments were performed on particle distribution in the high velocity wind tunnels after the new atomizer nozzles were installed to reduce the airborne particle size within the tunnels. Previous work on the AMP II Program had revealed that at high wind velocities, i.e., 30-35 mph, particles as large as 230 microns in diameter are capable of remaining airborne. In an effort to reduce the particle size, new nozzles were installed and tested by means of aluminum membranes which were placed in the wind tunnels and subjected to air stream flows of 30 miles per hour for one hour before being examined microscopically.

Table 4.1 shows the particle size distribution observed by microscopic examination. Particles as small as five microns in diameter were readily discernable microscopically. Below that size,

LASER DRILLED MEMBRANE HOLE





1 mm = 13.3μ

PHOTOMICROGRAPH P-2

TABLE 4.1

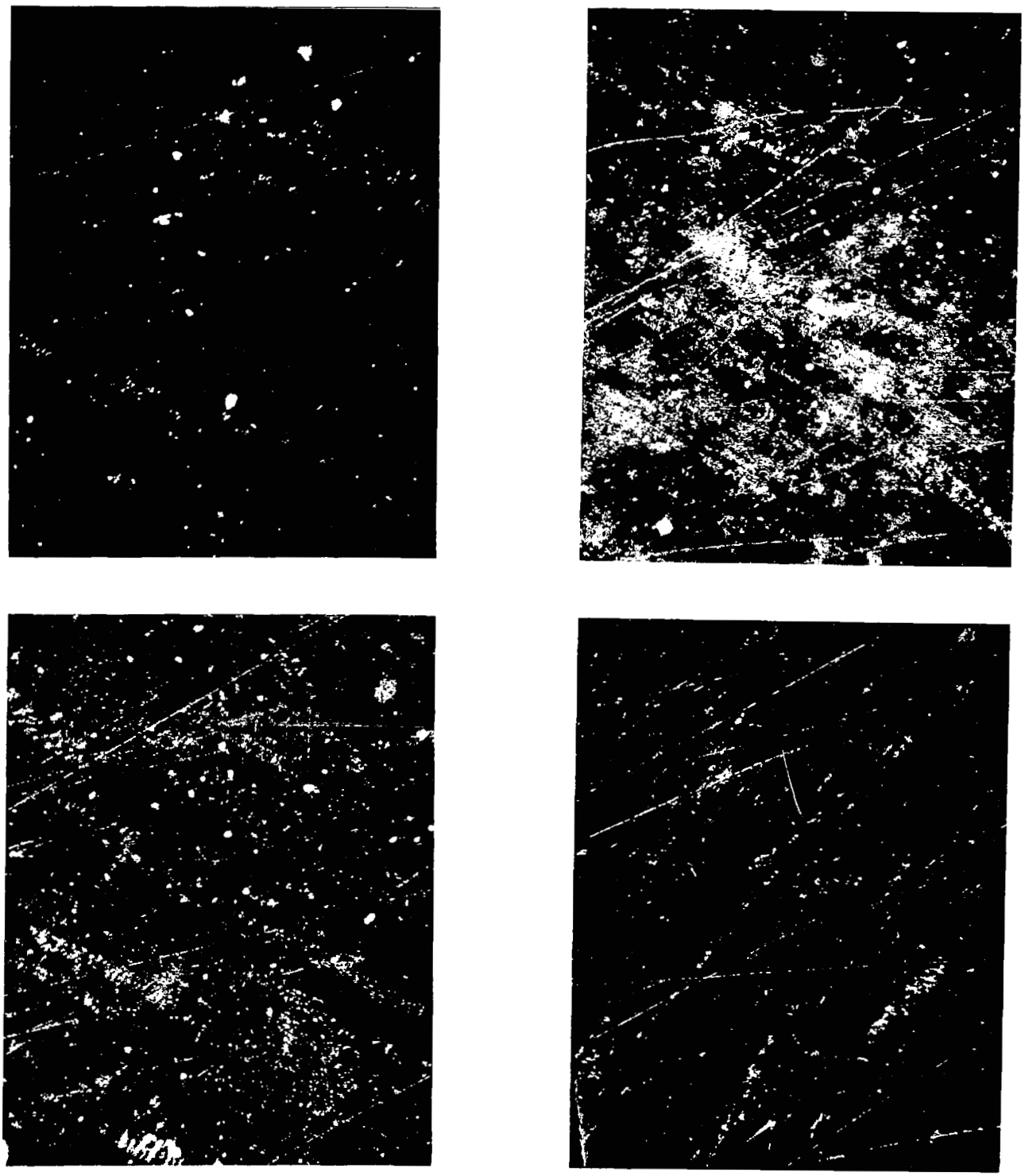
HIGH VELOCITY PARTICLE SIZE DISTRIBUTION AT 30 MPH

Particle Diameter	No. Counted	% of Total
5-10	218	61%
10-20	121	34%
20-30	21	5%
30	0	0%
	360	100%

it became impossible to differentiate between bacterial particles and the extraneous scratches, nicks, etc. on the face of the aluminum membrane. The experimental results show that with the new nozzles, the particle sizes have been reduced by an order of magnitude.

Photograph number P-3 are photomicrographs of an aluminum membrane taken after an actual test run at 30 miles per hour. The photo in the lower right is that of a blank membrane included for comparison. An occasional 25 to 30 μ particle may be observed on the photographs. Most sizes, however, fall below 10 μ in diameter.

PARTICLE DISTRIBUTION - HIGH VELOCITY



30 MPH

1 mm = 13.3

PHOTOGRAPH P-3

5.0 RESULTS AND DISCUSSION

5.1 QUIESCENT PROGRAM

A total of 46 tests were performed during the quiescent phase of the program. Hole sizes ranging from 1000 microns to 3000 microns in diameter were employed at pressure differentials ranging from 0.05 inches of water above ambient, to 0.5 inches. Table 5.1 is a summary of the quiescent test runs. The use of oxyethylene docosanol wax as a water evaporator retardant allowed for the increase in membrane hole diameter from the previous maximum diameter of 1887 microns to that of the 3000 microns diameter. This was accomplished without undue evaporation of moisture from the surface of the agar and with the knowledge that the bacteria would grow on the agar/wax surface, if the hole in the membrane was penetrated by a bacterial contaminant.

The quiescent test results, as evidenced by the build-up of statistical data, show results consistent with the theoretical and experimental analyses of AMP II, which indicated that a pressure differential only slightly above ambient was all that was required to prevent penetration under ambient conditions. No anomalies were encountered during the course of the quiescent testing which would in any way alter or cast doubt on the results obtained. The pressure of 0.05 inches of water above ambient was chosen as the minimum pressure point because it represented the lowest reading that could be taken on the manometer with a high degree of accuracy. A pressure differential of 0.5 inches of water was found totally adequate in preventing penetration of bacterial particles under quiescent conditions.

5.2 HIGH VELOCITY PROGRAM

A total of 50 tests were run during the high velocity program, utilizing hole diameters of 20, 200 and 1000 microns in diameter at wind velocities ranging from 10 to 30 miles per hour. Pressures from 0.05 inches to 3.2 inches water pressure above ambient were employed in an effort to prevent penetration through the various diameter holes.

The results of these tests are recorded in Tables 5.2, 5.3, and 5.4; and shown graphically combined with data from AMP II in Figures 5.1, 5.2, and 5.3. Figure 5.4 is a composite of all three graphs.

TABLE 5.1

QUIESCENT TEST RESULTS

#	Hole Size	Δ P	Test Results
1	1000 micron	0.5 inches	Negative
2	1000	0.5	Negative
3	1000	0.5	Negative
4	1000	0.5	Negative
5	1000	0.5	Negative
6	1667	0.5	Negative
7	1667	0.5	Negative
8	1667	0.5	Negative
9	1887	0.5	Negative
10	1887	0.5	Negative
11	1887	0.5	Negative
12	1000	2.0	Negative
13	1000	2.0	Negative
14	1000	2.0	Negative
15	1000	2.0	Negative
16	1000	2.0	Negative
17	1000	2.0	Negative
18	1000	2.0	Negative
19	1000	2.0	Negative
20	1000	2.0	Negative
21	1000	2.0	Negative
22	2000	0.5	Negative
23	2000	0.5	Negative
24	2000	0.5	Negative
25	2000	0.5	Negative
26	2000	0.5	Negative
27	2000	0.5	Negative
28	2000	0.5	Negative
29	2333	0.5	Negative
30	2333	0.5	Negative
31	2333	0.5	Negative
32	2333	0.5	Negative
33	2333	0.5	Negative
34	2333	0.5	Negative
35	2333	0.5	Negative
36	2333	0.5	Negative

TABLE 5.1 (CONTINUED)

QUIESCENT TEST RESULTS

#	Hole Size	ΔP	Test Results
37	3000 micron	0.5 inches	Negative
38	3000	0.5	Negative
39	3000	0.5	Negative
40	3000	0.5	Negative
41	3000	0.5	Negative
42	3000	0.5	Negative
43	3000	0.05	Negative
44	3000	0.05	Negative
45	3000	0.05	Negative
46	3000	0.05	Negative

TABLE 5.2

HIGH VELOCITY TEST RESULTS

20 MICRON HOLES

MPH	ΔP	TEST RESULTS
10	.05	Negative
10	.05	Negative
10	.05	Negative
10	.05	Negative
15	.05	Negative
15	.05	Negative
15	.05	Negative
15	.05	Negative
15	.05	Negative
20	.05	Negative
20	.05	Negative
20	.05	Negative
20	.05	Negative
25	.05	Negative
25	.05	Negative
25	.05	Negative
25	.05	Negative
25	.05	Negative
30	.05	Negative
30	.05	Negative
30	.05	Negative

TABLE 5.3

HIGH VELOCITY TEST RESULTS

200 MICRON HOLES

MPH	ΔP	TEST RESULTS
10	.05	Positive
10	.05	Positive
10	.075	Positive
10	.10	Negative
15	.05	Positive
15	.1	Negative
15	.1	Negative
15	.1	Negative
20	.05	Positive
20	.05	Negative
20	.10	Negative
20	.10	Negative
20	.10	Negative
25	.05	Positive
25	.05	Positive
25	.10	Positive (1 colony)
25	.10	Negative
25	.10	Negative
25	.10	Negative
25	.10	Negative
30	.20	Negative
30	.20	Negative
30	.50	Negative
30	.50	Negative

TABLE 5.4

HIGH VELOCITY TEST RESULTS

1000 MICRON HOLES

MPH	ΔP	TEST RESULTS
20	0.5	Positive
20	0.5	Negative
20	0.5	Negative
25	1.2	Positive
25	1.2	Negative
25	1.5	Negative
30	2.4	Positive
30	3.0	Positive
30	3.0	Negative
30	3.2	Negative
30	3.2	Negative

HIGH VELOCITY RESULTS
20 μ HOLE DIAMETER

EFFECT OF IMPINGING AIR STREAM VELOCITY ON MICROBIAL PENETRATION THROUGH MICROSCOPIC HOLES IN MEMBRANES

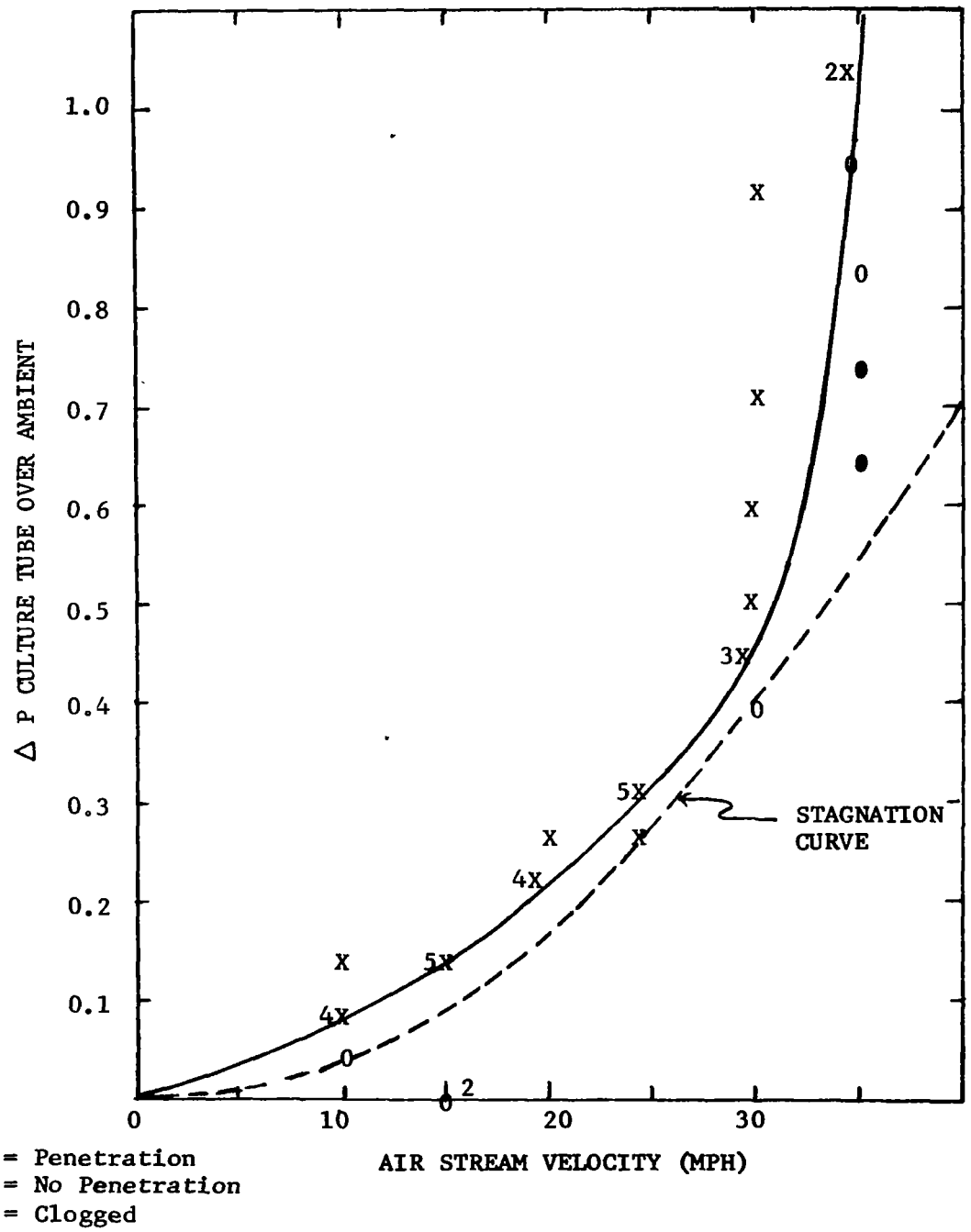


FIGURE 5.1

HIGH VELOCITY RESULTS
200 μ HOLE DIAMETER

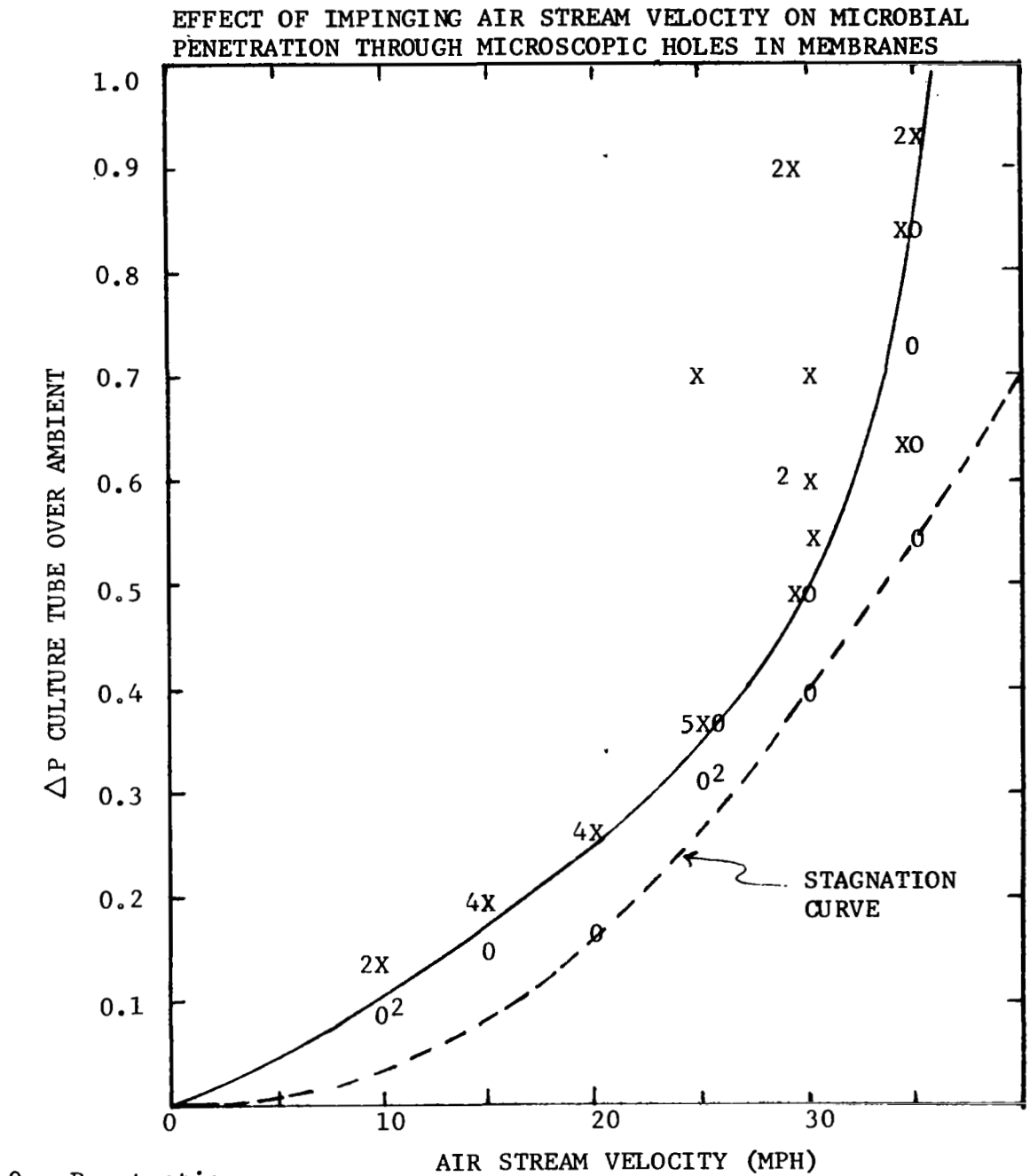
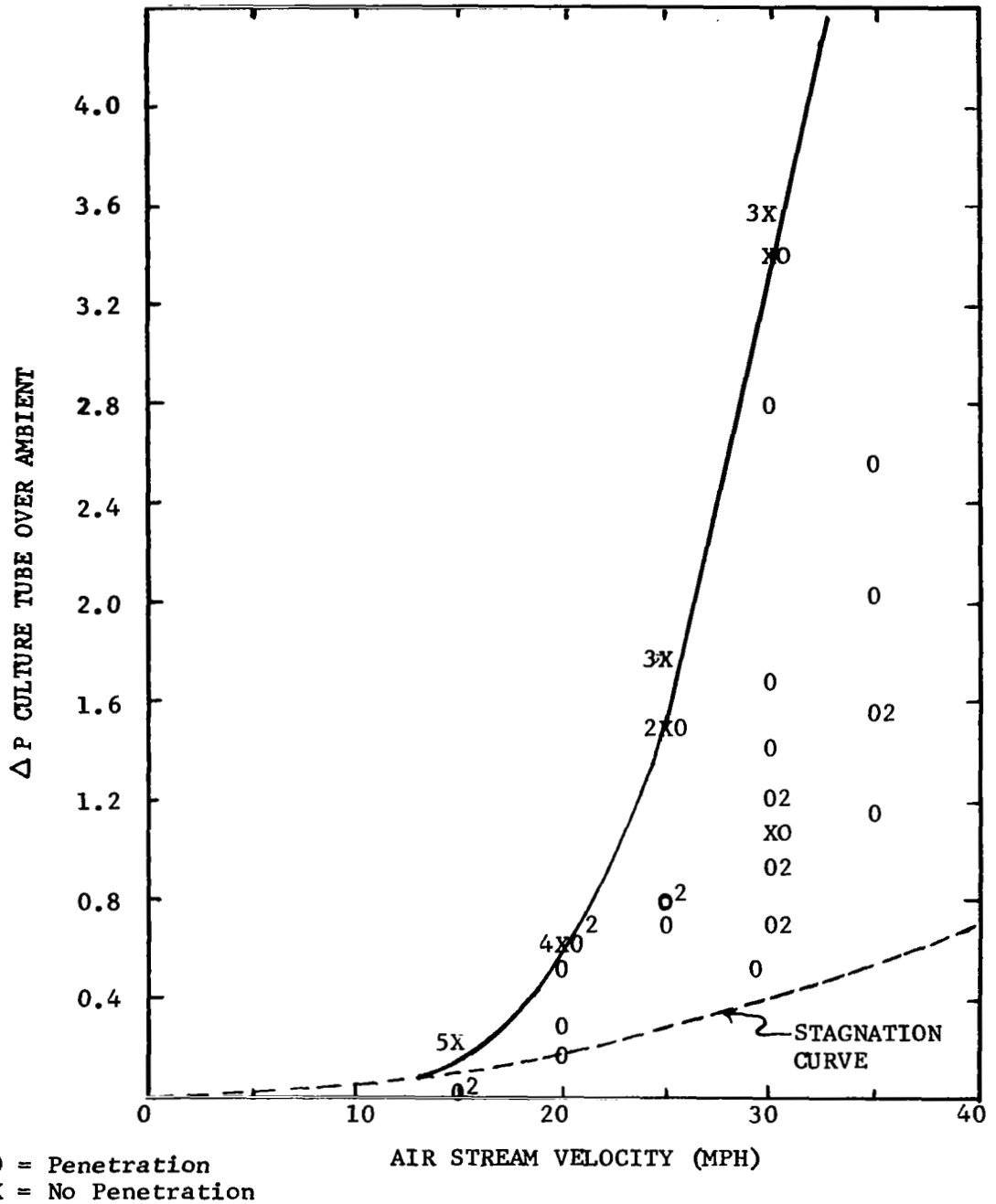


FIGURE 5.2

HIGH VELOCITY RESULTS
1000 μ HOLE DIAMETER

EFFECT OF IMPINGING AIR STREAM VELOCITY OF MICROBIAL PENETRATION THROUGH MICROSCOPIC HOLES IN MEMBRANES

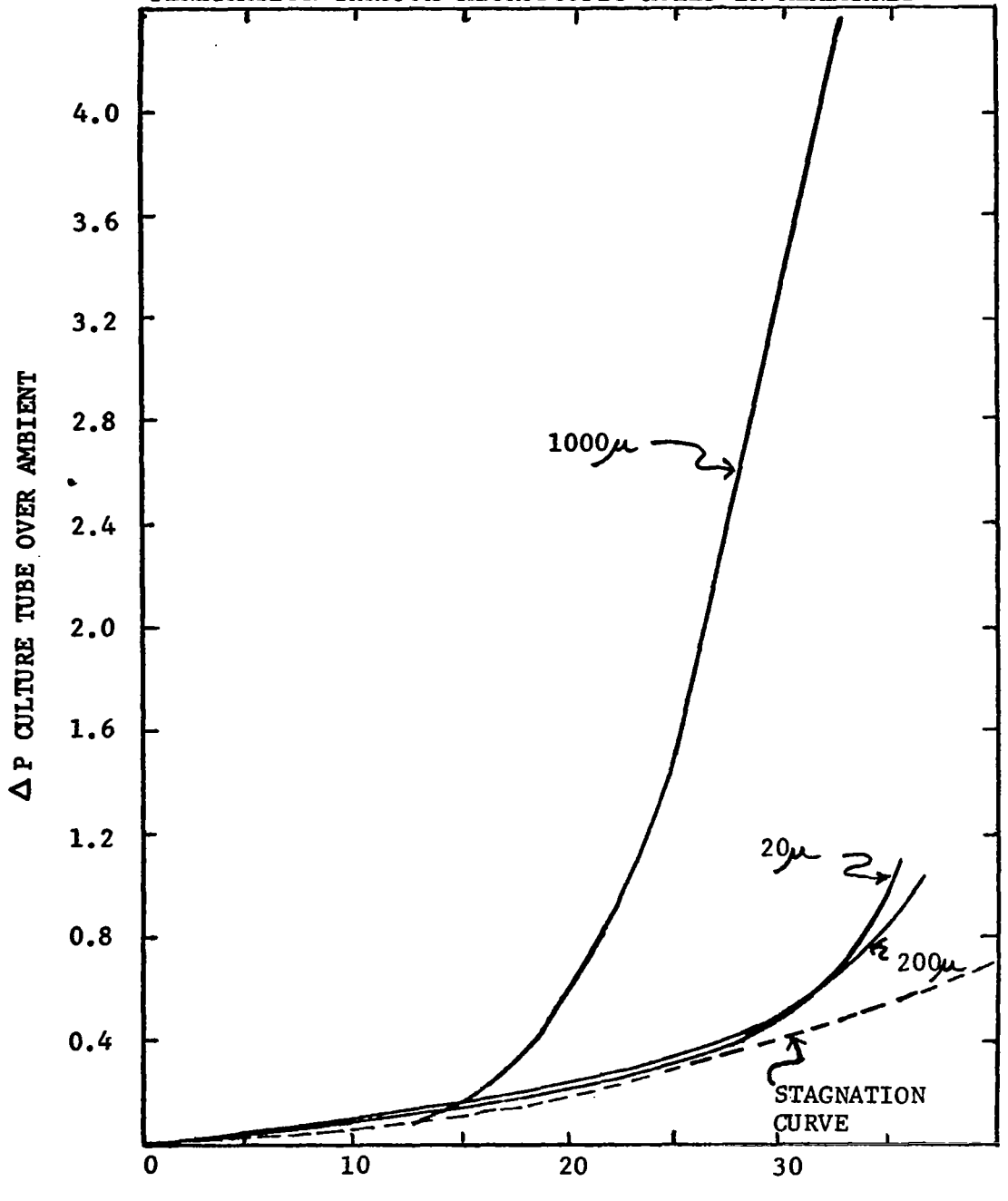


0 = Penetration
X = No Penetration

FIGURE 5.3

COMPOSITE RESULTS - HIGH VELOCITY

EFFECT OF IMPINGING AIR STREAM VELOCITY OF MICROBIAL PENETRATION THROUGH MICROSCOPIC HOLES IN MEMBRANES



COMPOSITE

FIGURE 5.4

From Figure 5.1 it becomes apparent that for wind velocities up to 30 mph, a water pressure of slightly above 0.4 inches over ambient was all that was required to prevent penetration of all the airborne particles that were observed by the 20 μ hole diameter. As the air velocity increases beyond 30 miles per hour, the Δ P needed to effectively prevent penetration rises sharply, i.e., 1.05 inches at 35 mph. Figure 5.2 shows the effects of wind velocity on a 200 micron hole diameter. Initially, sporatic penetration was observed at 0.05 inch differential pressure above stagnation. As the Δ P was increased to 0.1 inch however, the increase effectively prevented penetration at velocities up to 25 miles per hour. This corresponds to a Δ P above ambient of approximately 0.36 inches water pressure. Beyond this critical velocity of 25 mph, the Δ P required rises sharply - i.e., 0.6 inches over ambient for particles traveling at velocities approaching 30 mph.

The one positive culture tube (there were four negative tubes) observed during the test runs at 25 mph and 0.36 Δ P over ambient had only one colony growing on the culture agar after 72 hours of testing. This was reported as a violation of sterility and could be attributed to a particle larger than those previously observed, presenting itself to the hole at a velocity of 25 mph and penetrating the outflowing air, regulated to 0.36 inches above ambient. This was the only violation of sterility at that particular pressure and velocity and lends credence to the belief that the system employed is potentially sensitive to the penetration of one particle.

Figure 5.3 shows the effects of particles being presented to a hole approximately 1000 microns in diameter. In total, eleven tests were performed at wind velocities of 20, 25 and 30 miles per hour. These results, together with those of the previous work, establishes a critical velocity for the 1000 micron diameter hole, at about 15 miles per hour. Beyond this velocity, the chance of penetration becomes highly sensitive to the particle velocity and hole diameter. Figure 5.4 shows a composite of the three graphs.

At no time during the course of the high velocity testing was it necessary to shut down equipment due to a mechanical failure. This could be attributed to the painstaking designs which were incorporated into the AMP II program. The only change, as mentioned previously, was the new aerosol dispenser nozzle incorporated into the wind tunnels prior to the test runs. The fact that no additional changes were necessary makes it possible to incorporate the AMP II results with the results obtained during the course of these experiments.

5.3 QUIESCENT - LOSS OF PRESSURE

A total of 35 tests were performed with the modified quiescent apparatus in an effort to determine the minimum time required for microbial penetration on the occasion of loss of pressure differential. The results of these experiments are shown in Table 5.5. The sensitivity of the instrumentation was such that a time lapse as small as 0.2 seconds after the pressure decay reached ambient, could be recorded. In one instance in which a 1000 micron hole was employed, a 1.0 second down-time was all that was required to allow penetration. All the tests that were run in such a way as to allow the pressure to return to 2.0 inches one second before ambient was reached, showed no penetration through the hole. Indeed, those tests in which the pressure decay reached ambient and were immediately returned to 2 inches without a recordable time delay, resulted in a failure for the microorganisms to penetrate. Only during the test runs where a recordable time delay (0.2 seconds or greater) was allowed after the system reached ambient, was penetration observed. In most instances this time delay was in the order of 1 second. With the 20 micron diameter hole, however, no penetration occurred after a delay of up to 10 seconds. It was only when the delay was extended to 30 seconds that penetration was observed. As previously mentioned, this phenomena may have been due to the organisms not presenting themselves to the 20 μ hole during the time lapse, or, the pressure decay may not have reached ambient after 8.5 minutes, but may have been at some point between .005 inches Δ P, and zero. Figures 5.5, 5.6, and 5.7 show the results of the preliminary work in establishing the pressure drop/time curves.

TABLE 5.5

QUIESCENT TESTS WITH PRESSURE DROP

TEST #	HOLE SIZE	TIME (SEC)*	PENETRATION
1	20	+1	Negative
2	20	+1	Negative
3	20	+10	Negative
4	20	0	Negative
5	20	+10	Negative
6	20	+30	Positive
7	20	+60	Positive
8	100	+1	Positive
9	100	+1	Positive
10	100	+1	Positive
11	100	+1	Positive
12	100	-1	Negative
13	100	0	Negative
14	100	-1	Negative
15	100	-1	Negative
16	100	+3	Positive
17	100	+2	Positive
18	100	+1	Positive
19	200	+1	Positive
20	200	+1	Positive
21	200	+1	Positive
22	200	+1	Positive
23	200	-1	Negative
24	200	-1	Negative
25	200	0	Negative
26	200	0	Negative
27	200	0	Negative
28	1000	+1	Positive
29	1000	0	Negative
30	1000	0	Negative
31	1000	+.8	Positive
32	1000	-.2	Negative
33	1000	+.2	Positive
34	1000	-.2	Negative
35	1000	+.2	Positive

* + indicates time in seconds after zero Δ P.
- indicates time in seconds before zero Δ P.
0 indicates time when pressure reached zero Δ P.

33 μ HOLE PRESSURE DECAY

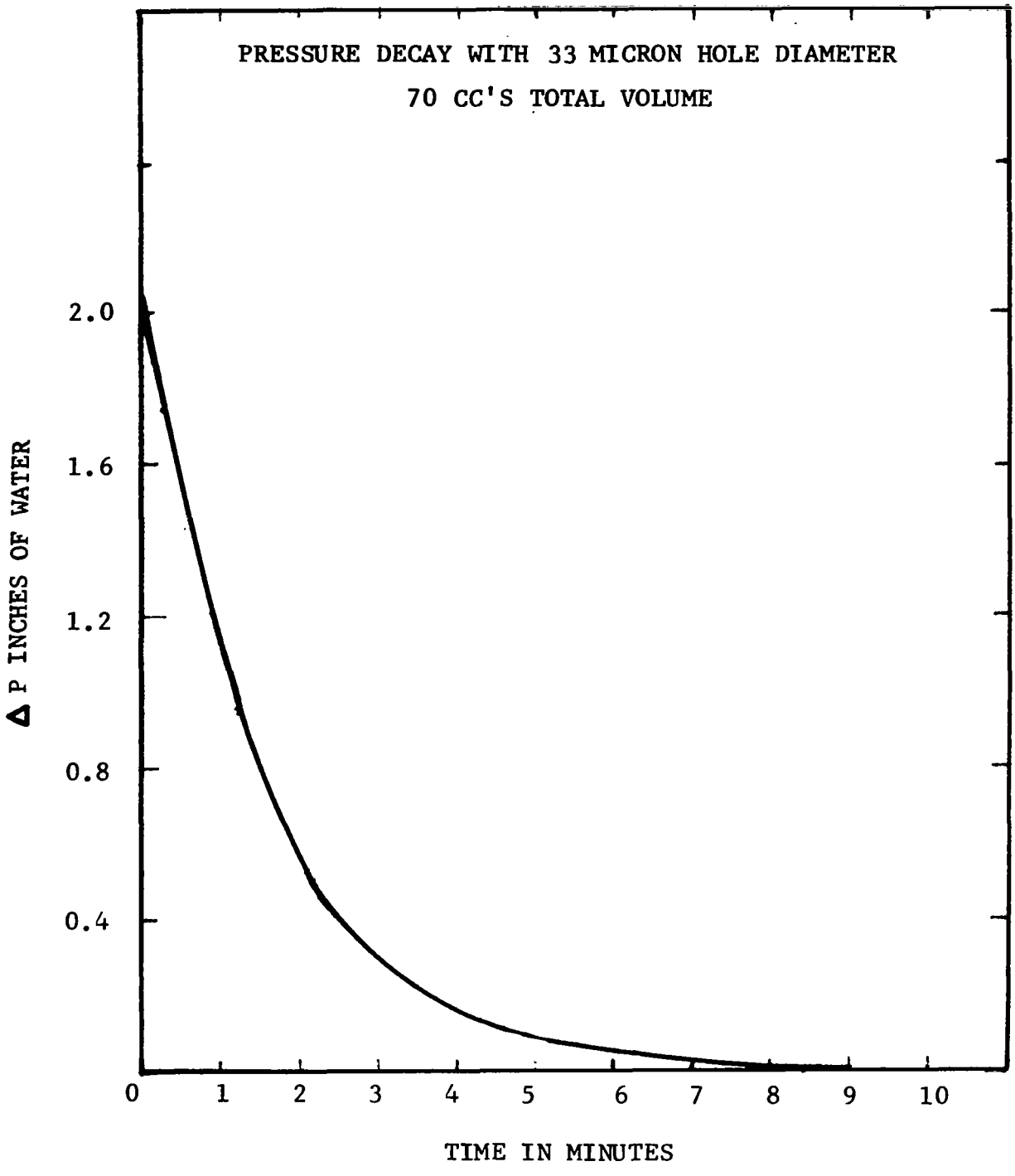


FIGURE 5.5

220 μ HOLE PRESSURE DECAY

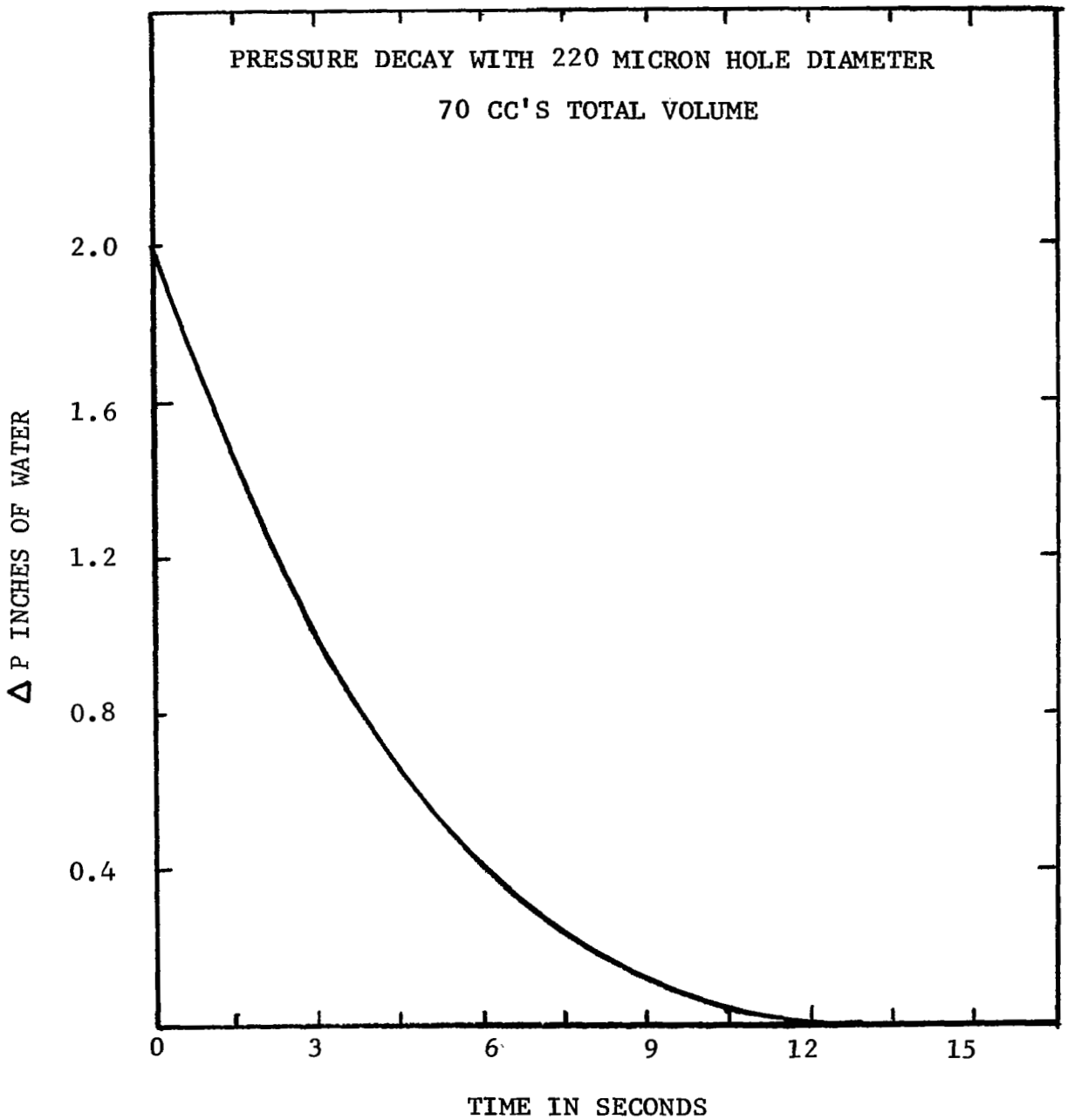


FIGURE 5.6

1000 μ HOLE - PRESSURE DECAY

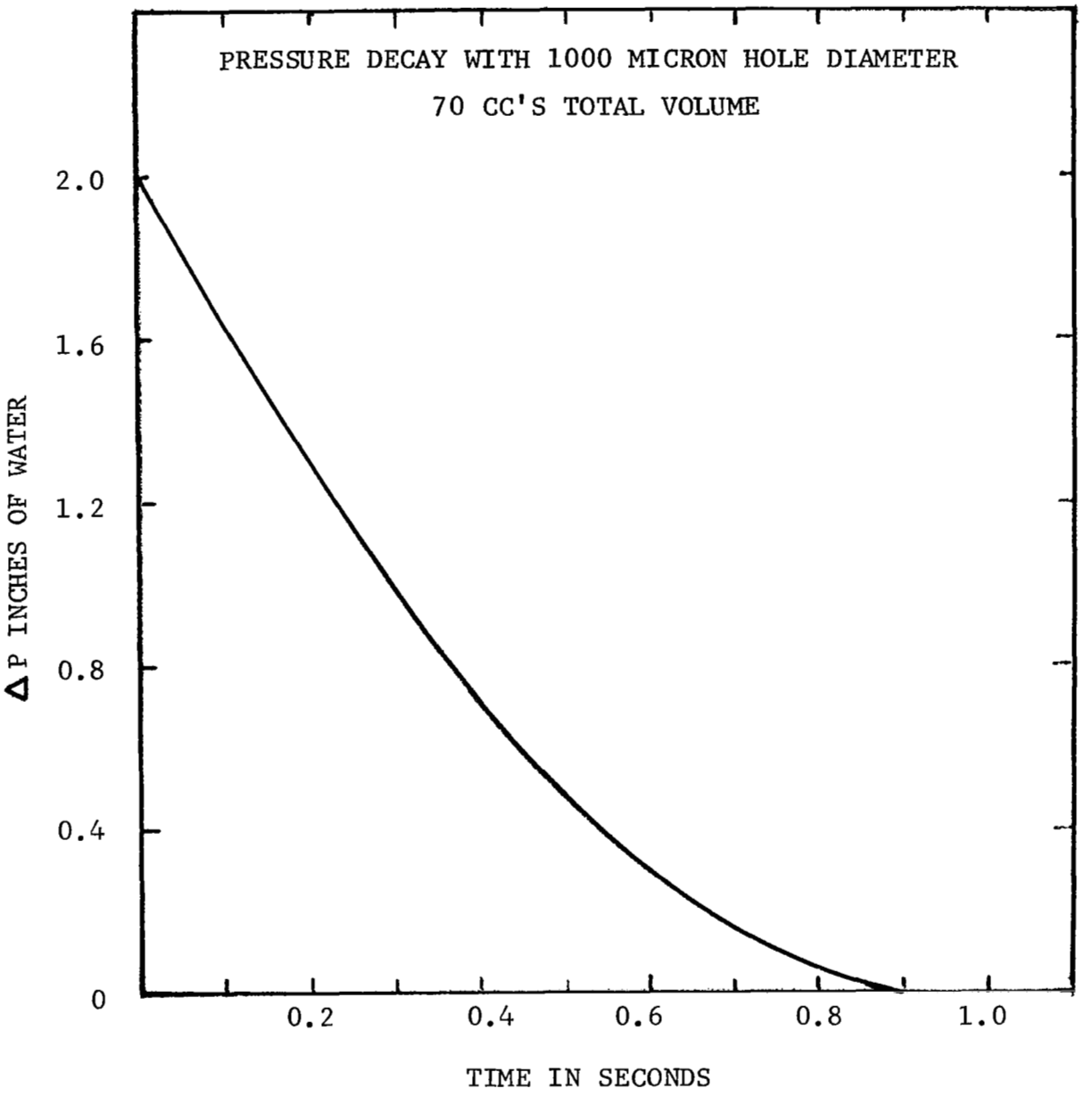


FIGURE 5.7

6.0 CONCLUSIONS

The test data accumulated during the course of the three AMP programs provides a foundation upon which one can build a probability of recontamination evaluation. All one needs for such a statistical evaluation for a program such as Viking, is the necessary environmental data which the capsule encounters.

The experimental and analytical data accrued shows that the techniques employed during the course of the contract were reliable. Accumulated data indicates that the systems employed are potentially sensitive to penetration by 1 of the 10^8 viable particles that were aerosoled into the test chamber or wind tunnel during a typical test run. The microorganism(s) upon transgressing the hole would be deposited upon the culture media previously treated to retard evaporation, and during the course of the 72 hour test run, would grow into a colony(s) large enough to be seen upon visual examination. Safeguards were built into the system so that growth on the agar within the viable culture chamber could only be the result of penetration through the hole in the test membrane in question.

6.1 QUIESCENT PROGRAM

Test data from the quiescent program revealed no dependence of penetration on particle size and hole size with regard to spore particles up to 35 microns in diameter, and hole sizes up to 3000 microns in diameter. It is clearly evident that under quiescent conditions, a pressure differential of 0.5 inches of water is more than adequate to prevent penetration by viable particles up to 35 microns in diameter. Theoretical analysis of this situation indicates that 0.5 inches of water should stop penetration of particles up to 200 microns in diameter for hole sizes up to several thousand microns in diameter. The 0.5 inch of water pressure differential, then, would appear to be a conservative pressure level, keeping in mind, however, that a series of four tests with the 3000 μ hole diameter proved negative (no penetration) with as little as 0.05 inches of water pressure differential.

6.2 HIGH VELOCITY PROGRAM

Both experimental and theoretical data pertaining to the high velocity program, indicate a growing dependence on hole size, particle size, and air velocity. The data also indicate that any

practical application of the use of a positive pressure differential as a means of maintaining sterility must take into consideration the control of these three parameters - foremost of which appears to be the external air stream velocity. A pressure differential of 2.0 inches of water pressure above ambient would be adequate to prevent penetration of microbial particles, up to 200 microns in diameter, traveling at velocities up to 25 mph, through holes up to 1000 μ in diameter.

6.3 QUIESCENT PROGRAM - TRANSIENT PRESSURE LOSS

The testing program on transient pressure loss has clearly indicated that a pressure drop to ambient sustained for as short a time interval as one second constitutes a violation in the integrity of the sterility the system was designed to maintain.

APPENDIX A

AIR VELOCITY CALCULATIONS

The air velocity within the wind tunnel was measured by means of a pitot tube located near the beginning of the high velocity leg. This was connected to a pressure transducer gage which read velocity pressure in inches of water. Using this reading and utilizing a standard air density reading of 0.075 lbs/ft^3, the velocity in feet per minute was determined on an air velocity calculator slide rule, F.W. Dwyer Manufacturing Company, Michigan City, Indiana. From this reading, the miles per hour were determined.

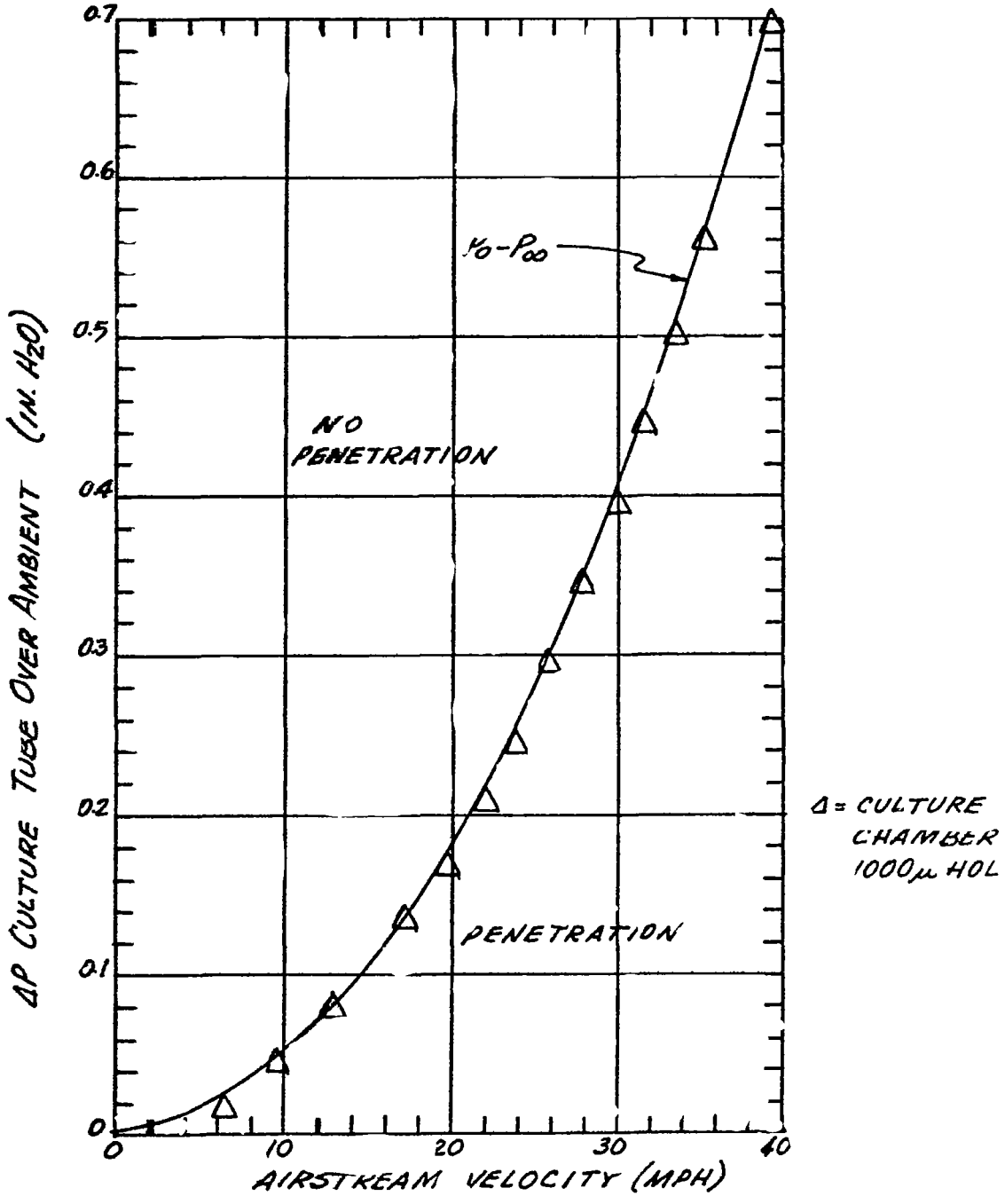
CONVERSION TABLE PITOT TUBE

Δ P TO MPH

VELOCITY			Δ P PITOT TUBE
10 mph	14.7 ft/sec	882 ft/min	0.049"
20 mph	29.3 ft/sec	1760 ft/min	0.19"
25 mph	36.7 ft/sec	2200 ft/min	0.30"
30 mph	44 ft/sec	2640 ft/min	0.43"
35 mph	51 ft/sec	3060 ft/min	0.58
40 mph	58.7 ft/sec	3520 ft/min	0.78"
45 mph	66 ft/sec	3960 ft/min	0.98"
50 mph	73.3 ft/sec	4400 ft/min	1.24"
60 mph	88 ft/sec	5280 ft/min	1.75"

APPENDIX B

EFFECT OF IMPINGING AIR STREAM VELOCITY ON MICROBIAL PENETRATION THROUGH MICROSCOPIC HOLES IN MEMBRANES - THEORETICAL



APPENDIX C-1

QUIESCENT TEST RESULTS

COMPOSITE RESULTS - AMP II AND III

NUMBER OF TESTS	HOLE SIZE	PRESSURE	TEST RESULTS
2	20	.00	+
12	20	.50	-
2	200	.00	+
1	200	.50	+
11	200	.50	-
2	1000	.00	+
20	1000	.50	-
16	1000	2.00	-
3	1667	0.50	-
3	1887	0.50	-
7	2000	0.50	-
8	2333	0.50	-
4	3000	0.05	-
6	3000	0.50	-
97 Tests			

\+ = Penetration

\- = No Penetration

APPENDIX C-2

HIGH VELOCITY RESULTS

COMPOSITE RESULTS - AMP II AND III

20 μ HOLE DIAMETER

MPH	10		15		20		25		30		35	
	ΔP		ΔP		ΔP		ΔP		ΔP		ΔP	
	.00	+	.00	+			.00	+	.00	+		
			.00	+								
	.05	-	.05	-	.05	-	.05	-	.05	-		
	.05	-	.05	-	.05	-	.05	-	.05	-		
	.05	-	.05	-	.05	-	.05	-	.05	-		
	.05	-	.05	-	.05	-	.05	-				
							.05	-				
			.10	-	.10	-	.10	-			.10	+
											.10	⊕
									.20	-	.20	⊕
									.20	-		
									.30	-	.30	+
									.30	-		
									.30	-		
											.40	+
									.50	-	.50	-
											.50	-

+ = Penetration

- = No Penetration

⊕ = Clogged Orifice

APPENDIX C-3

HIGH VELOCITY RESULTS

COMPOSITE RESULTS - AMP II AND III

200 μ HOLE DIAMETER

MPH	10		15		20		25		30		35	
	Δ P		Δ P		Δ P		Δ P		Δ P		Δ P	
					.00	+			.00	+	.00	+
	.05	+	.05	+	.05	+	.05	+				
	.05	+			.05	-	.05	+				
	.075	+										
	.10	-	.10	-	.10	-	.10	+	.10	+	.10	+
	.10	-	.10	-	.10	-	.10	-	.10	-	.10	-
			.10	-	.10	-	.10	-				
					.10	-	.10	-				
							.10	-				
							.10	-				
									.15	-		
									.20	-	.20	+
									.20	-		
									.30	-	.30	-
									.50	-	.30	+
							.43	-	.50	-	.40	-
											.40	-

\+ = Penetration

\- = No Penetration

APPENDIX C-4

HIGH VELOCITY RESULTS

COMPOSITE RESULTS - AMP II AND III

1000μ HOLE DIAMETER

MPH 10	15	20	25	30	35
ΔP	ΔP	ΔP	ΔP	ΔP	ΔP
	.00 +	.00 +			
	.00 +				
	.10 -	.10 +		.10 +	
	.10 -				
	.10 -				
	.10 -				
	.10 -				
		.30 +		0.30 +	
				0.30 +	
			.40 +		
			.43 +		
		.50 +		0.50 +	
		.50 -		0.50 -	
		.50 -	.50 -		
			.53 -		
			.55 +	0.60 +	.60 +
				0.60 +	
				.80 +	
				.80 -	
			1.20 +	1.0 +	1.0 +
			1.20 -	1.3 +	1.0 +
			1.20 -		
			1.50 -		
			1.50 -		
			1.50 -		1.50 +
				2.4 +	2.00 +
				3.0 +	3.00 +
				3.0 -	
				3.2 -	
				3.2 -	
				3.2 -	

+ = Penetration

- = No Penetration

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